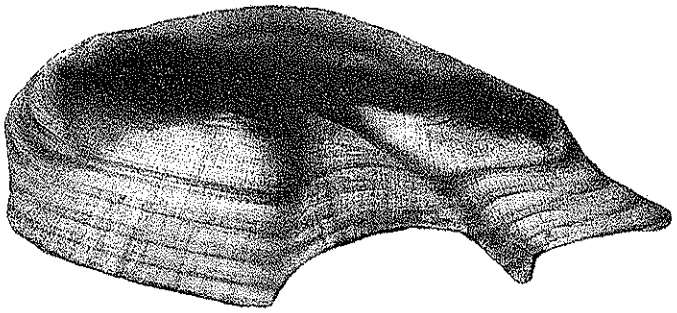
FINAL REPORT FOR 2006
VIRGINIA - CHESAPEAKE BAY FINFISH AGEING



by

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January 31, 2008

Final Report

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

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is completely opposite to previous studies which reported tautog as a slow-growing fish when the opercula-based ageing method was used. This finding will be presented in 2007 Age and Growth Report. Second, following the CCA initiative, in 2006 we started a research project on the sheepshead (*Archosargus probatocephalus*) population dynamics in the Chesapeake Bay funded by VMRC. This project will continue for next two years and its finding will be reported to VMRC.

As part of our continued public outreach focused at recreational anglers, we again participated in the CCA's Kid's Fishing Day at Lynnhaven Fishing Pier. This was the sixth year our staff volunteered their time to participate in the event. To support other environmental and wildlife agencies, and charities, we donated more than 5,000 pounds of dissected fish to a local wildlife rescue agency which is responsible for saving injured animals found by the public, and to the Salvation Army.

In 2006, we continue to upgrade our Age & Growth Laboratory website, which can be accessed at http://web.odu.edu/fish. The website includes electronic versions of this document along with more detailed explanations of the methods and structures we use in age determination.

Table I. Summary of numbers aged and age ranges for the 13 marine fish species collected for age determination in Virginia during 2006.

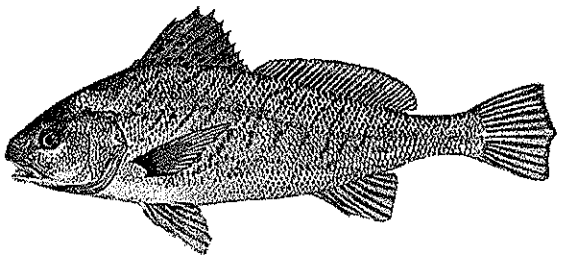
Species	Number of Fish	Number of Hard-Parts	Number of Age Readings	Minimum Age	Maximum Age
Atlantic croaker	339	339	778	2	15
black drum	9	9	27	0	4
bluefish	323	323	746	0	12
cobia	29	29	87	3	16
red drum	16	16	48	1	3
spadefish	326	326	752	0	19
Spanish mackerel	291	291	682	1	8
spot	263	263	626	0	4
spotted seatrout	256	256	612	1	4
striped bass	913	1247	2694	2	20
summer flounder	871	1367	2934	1	11
tautog	503	975	2150	2	14
weakfish	641	641	1382	1	5
Totals	4780	6082	13518		

Acknowledgements

We thank Mrunalini Chitty, Billy Culver, Meredith McPherson, and Sonya Phillip 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 also thankful for Dr. William Persons III hard work on our *Species Updates* and web page. A special note of appreciation to Ron Owens, Troy Thompson, Joanie Beatley, and Myra Thompson, and Tara Bushnoe for their many efforts in this cooperative project. We would like also to thank our Ph.D. students Joey Ballenger and Stacy Beharry, and Postdoc 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 a 315 mm (12.4 inch) total length, 5 year-old male spot. The fifth annulus is forming at the edge of the otolith.

Chapter 1
Atlantic Croaker



Micropogonias undulatus

INTRODUCTION

A total of 339 Atlantic croaker, *Micropogonias undulatus*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. The average age was 6.2 years, and the standard deviation and standard error were 2.42 and 0.13, respectively. Thirteen age classes (2 to 12 and 14 to 15) were represented, comprising fish from the 1991-1992, 1994-2004 year-classes. Fish from the 2001 year-class dominated the sample.

METHODS

Handling of collection — 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 labeled cell well plates.

Preparation — Otoliths were processed following the methods described in Barbieri et al. (1994) with a few modifications. Briefly, the left or right sagittal otolith was randomly selected and attached to a glass slide with Aremco's clear Crystalbond™ 509 adhesive. At least two serial transverse sections were cut through the core of each otolith with a Buehler Isomet low-speed saw equipped with a three inch, fine grit Norton diamond-wafering blade. Otolith 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

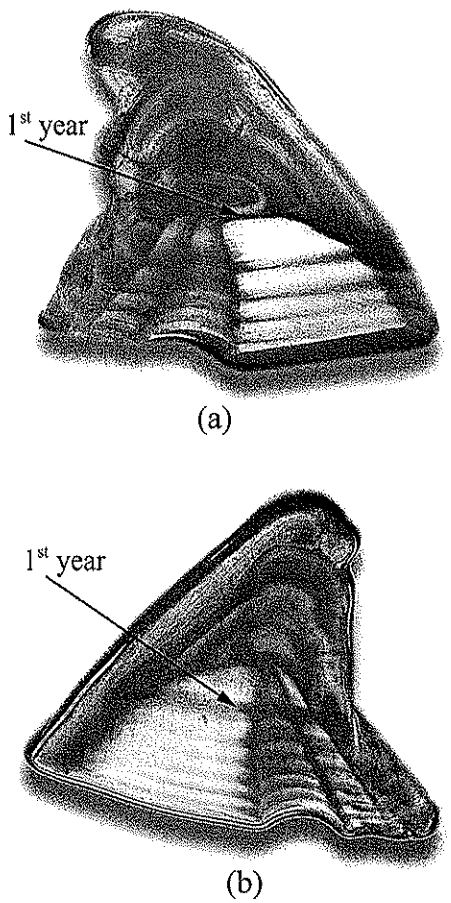


Figure 1. Otolith cross-sections of a) a 5 year old croaker with a small 1st annulus, and b) a 6 year old croaker with a large 1st annulus.

readability by increasing light transmission through the sections.

Readings — Sectioned otoliths were aged by two different readers using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples. The ageing criteria reported in Barbieri et al. (1994) were used in age determination, particularly regarding the location of the first annulus (Figure 1).

All samples were aged in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2003. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2003 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was very high for both readers (Reader 1's CV = 0.3% and Reader 2's CV = 0). There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 6$, df = 6, $P = 0.4232$). Figure 2 illustrates the between readers' precision of age estimates. There was also 97.6 percent agreement with an average CV of 0.3% between reader age estimates. There was no evidence of drift in age determination from Year 2003 precision fish with 100% agreement for Reader 1 and 98% agreement for Reader 2 (CV = 0.1%, test of symmetry: $\chi^2 = 1$, df = 1, P = 0.1373).

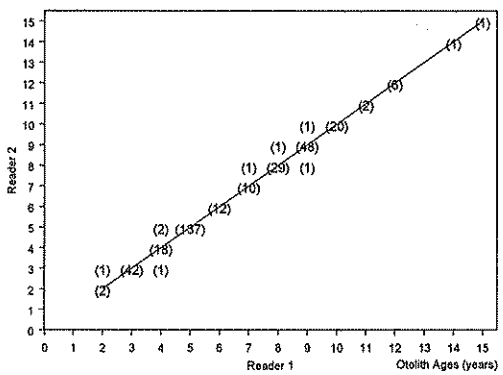


Figure 2. Between-reader comparison of otolith age estimates for Atlantic croaker in 2006

Of the 336 fish aged with otoliths, 13 age classes (2 to 12 and 14 to15) were represented (Table 1). The average age for the sample was 6.2 years, and the standard deviation and standard error were 2.42 and 0.13, respectively.

Year-class data (Figure 3) indicate that recruitment into the fishery begins at age 2, but large numbers are not seen until age 3,

which corresponds to the 2003 year-class for Atlantic croaker collected in 2006. The ratio of males to females shows an overall higher number of females, due to high abundance of females in 2001 year-class.

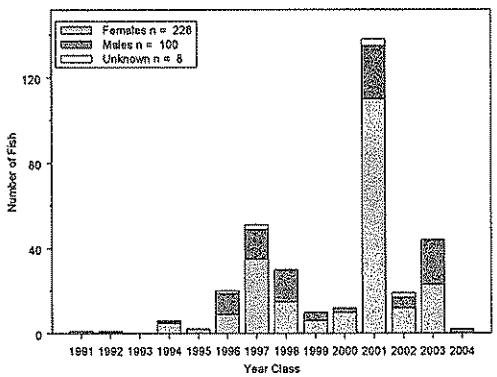


Figure 3. Year-class distribution for Atlantic croaker collected for ageing in 2006. Distributions are broken down by sex.

Age-Length-Key — In Table 2 we present an age-length-key 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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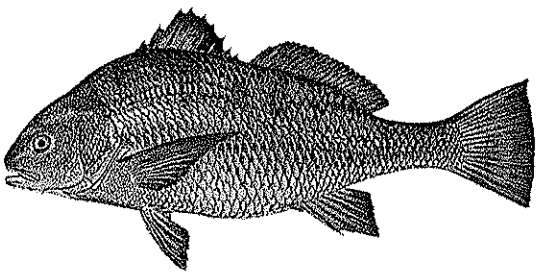
Table 1. The number of Atlantic croaker assigned to each total length-at-age category for 336 fish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)													
	2	3	4	5	6	7	8	9	10	11	12	14	15	Totals
9 - 9.99	1	13	0	0	0	0	0	0	0	0	0	0	0	14
10 - 10.99	0	20	1	1	0	0	0	0	0	0	0	0	0	22
11 - 11.99	1	10	6	17	2	0	0	0	0	0	0	0	0	36
12 - 12.99	0	1	8	43	4	1	1	5	2	1	1	0	0	67
13 - 13.99	0	0	2	32	5	5	3	3	2	0	0	0	0	52
14 - 14.99	0	0	1	28	1	0	6	10	3	0	0	0	1	50
15 - 15.99	0	0	0	9	0	3	10	11	4	0	0	1	0	38
16 - 16.99	0	0	0	5	0	0	7	7	6	0	1	0	0	26
17 - 17.99	0	0	1	2	0	0	3	10	2	0	3	0	0	21
18 - 18.99	0	0	0	0	0	1	0	3	1	0	1	0	0	6
19 - 19.99	0	0	0	0	0	0	0	2	0	1	0	0	0	3
20 - 20.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Totals	2	44	19	138	12	10	30	51	20	2	6	1	1	336

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-class, based on otolith ages for Atlantic croaker sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)													
	2	3	4	5	6	7	8	9	10	11	12	14	15	N
9 - 9.99	0.071	0.929	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	14
10 - 10.99	0.000	0.909	0.045	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	22
11 - 11.99	0.028	0.278	0.167	0.472	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	36
12 - 12.99	0.000	0.015	0.119	0.642	0.060	0.015	0.015	0.075	0.030	0.015	0.015	0.000	0.000	67
13 - 13.99	0.000	0.000	0.038	0.615	0.096	0.096	0.058	0.058	0.038	0.000	0.000	0.000	0.000	52
14 - 14.99	0.000	0.000	0.020	0.560	0.020	0.000	0.120	0.200	0.060	0.000	0.000	0.000	0.020	50
15 - 15.99	0.000	0.000	0.000	0.237	0.000	0.079	0.263	0.289	0.105	0.000	0.000	0.026	0.000	38
16 - 16.99	0.000	0.000	0.000	0.192	0.000	0.000	0.269	0.269	0.231	0.000	0.038	0.000	0.000	26
17 - 17.99	0.000	0.000	0.048	0.095	0.000	0.000	0.143	0.476	0.095	0.000	0.143	0.000	0.000	21
18 - 18.99	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.500	0.167	0.000	0.167	0.000	0.000	6
19 - 19.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.667	0.000	0.333	0.000	0.000	0.000	3
20 - 20.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
21 - 21.99	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
											Sample Size			336

Chapter 2
Black Drum



Pogonias cromis

INTRODUCTION

A total of 9 black drum, *Pogonias cromis*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. The average age of the sample was 2 years, with a standard deviation of 1.5 and a standard error of 0.5. The youngest fish was 0 year old and the oldest fish was 4 years old, representing the 2002, 2003, 2005, and 2006 year-classes, respectively.

METHODS

Handling of collection — 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 sample number. All otoliths were stored dry in their original VMRC coin envelopes.

Preparation — Otoliths were processed for ageing following the methods described in Bobko (1991) and Jones and Wells (1998). Briefly, at least two serial transverse sections were cut through the nucleus of each otolith with a Buehler Isomet low-speed saw equipped with a three inch, fine grit Norton diamond-wafering blade. Otolith 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 — Sectioned otoliths were aged by two different readers using a Leica MZ-12 dissecting microscope with transmitted light at between 8 and 20 times magnification (Figure 1). Each reader aged all of the otolith samples.

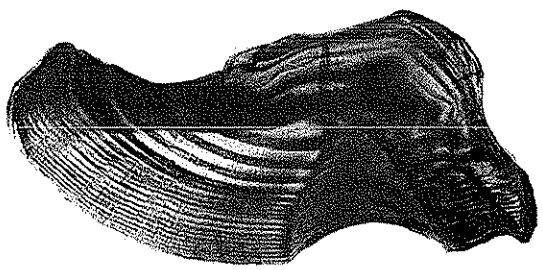


Figure 1. Otolith thin-section from a 20 year-old black drum.

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, 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 — Reader 1 aged all fish for second time to measure reader

precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Measurements of reader self-precision were very high, with Reader 1 able to reproduce 100 % of the ages of previously read otoliths. There was also 100 percent agreement between reader age estimates. Figure 2 illustrates the between readers' precision of age estimates. There was no evidence of drift in age determination from Year 2000 precision fish. Agreements for one year or less were 98% for Read 1 (CV = 0.7%, test of symmetry: $\chi^2 = 13$, df = 13, P = 0.4478) and 90% for Read 2 (CV = 1.6%, test of symmetry: $\chi^2 = 18.3$, df = 20, P = 0.5655).

Of the 9 fish aged with otoliths, 4 age classes were represented (Table 1). The average age of the sample was 2 years, with a standard deviation of 1.5 and a standard error of 0.5. The youngest fish was a 0 year old and the oldest fish was 4 years old, representing the 2002, 2003, 2005, and 2006 year-classes, respectively (Figure 3).

Age-Length-Key — In Table 2 we present an age-length-key that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

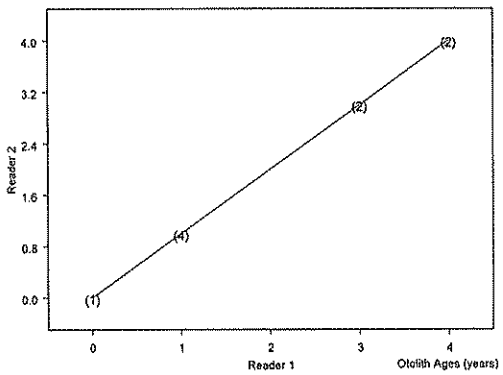


Figure 2. Between-reader comparison of otoliths age estimates for black drum in 2006.

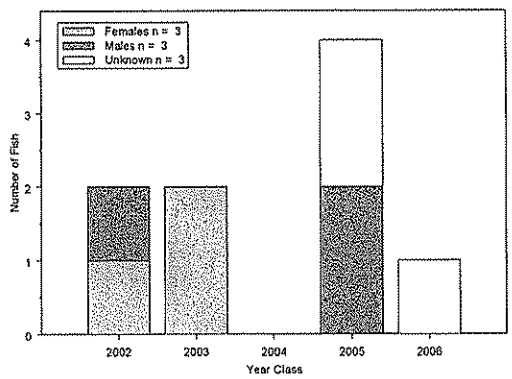


Figure 3. Year-class distribution for black drum collected for ageing in 2006. Distributions are broken down by sex.

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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.

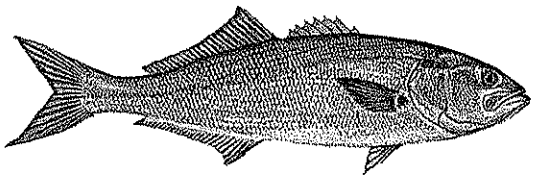
Table 1. The number of black drum assigned to each total length-at-age category for 9 fish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)				
	0	1	3	4	Totals
8 - 8.99	1	0	0	0	1
11 - 11.99	0	2	0	0	2
17 - 17.99	0	1	0	0	1
18 - 18.99	0	1	0	0	1
20 - 20.99	0	0	1	0	1
22 - 22.99	0	0	1	0	1
24 - 24.99	0	0	0	1	1
25 - 25.99	0	0	0	1	1
Total	1	4	2	2	9

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-intervals, based on otolith ages for black drum sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)				
	0	1	3	4	N
8 - 8.99	1.000	0.000	0.000	0.000	1
11 - 11.99	0.000	1.000	0.000	0.000	2
17 - 17.99	0.000	1.000	0.000	0.000	1
18 - 18.99	0.000	1.000	0.000	0.000	1
20 - 20.99	0.000	0.000	1.000	0.000	1
22 - 22.99	0.000	0.000	1.000	0.000	1
24 - 24.99	0.000	0.000	0.000	1.000	1
25 - 25.99	0.000	0.000	0.000	1.000	1
				Samples Size	9

Chapter 3
Bluefish



Pomatomus saltatrix

INTRODUCTION

A total of 332 bluefish, *Pomatomus saltatrix*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. We were unable to age 9 fish due to the damage of their otoliths. The average age was 2.1 years, and the standard deviation and standard error were 1.78 and 0.10, respectively. Ten age classes (0 to 8 and 12) were represented, comprising fish from the 1994, 1998 to 2006 year-classes. The 2004 and 2005 year-classes dominated the sample.

METHODS

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 in labeled cell well plates.

Preparation — We used a bake and thin-section technique to process bluefish otoliths for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each otolith was mounted with Crystal Bond onto a standard microscope slide with its distal surface orientated upwards. Once mounted, a small mark was placed on the otolith surface directly above the otolith focus. The slide, with attached otolith, was then secured to an Isomet saw equipped with two diamond wafering blades separated by a 0.5 mm spacer, which was slightly smaller in diameter than the diamond blades. The otolith was positioned so that the wafering blades straddled each side of the otolith focus ink 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 section was placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400°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 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 — Two different readers using a LEICA MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification aged all sectioned otoliths (Figure 1). 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. Unfortunately both these landmarks had a tendency to become less prominent in older fish.

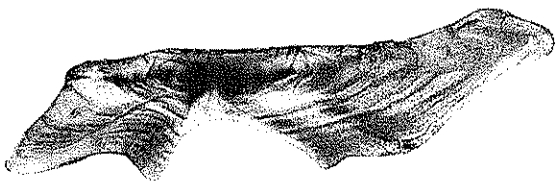


Figure 1. Otolith thin-section from a 850mm TL 8 year-old female bluefish.

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 four years of age and older. This annulus formation typically occurred within years 4 to 7, and was 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 origins being the sulcal groove and at the outer peripheral edge of the otolith. If these annuli did not meet to form a central origin they were considered two annuli, and 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a

reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was low for both readers (Reader 1's CV = 6.9% and Reader 2's CV = 11.9%). There was evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 24.01$, df = 11, $P = 0.0127$). Figure 2 illustrates the between readers' precision of age estimates. The average coefficient of variation (CV) of 8.3% was significant and lower than the CV of 13.7% in 2005. The between-reader agreement for otoliths for one year or less was 98.8% of all aged fish. Such a high agreement between the readers and the high CVs were partially due to the sample dominated by younger fish.

There was no evidence of drift in age determination from Year 2000 precision fish with 86% agreement for both readers. (Reader 1: CV = 13.6%, test of symmetry: $\chi^2 = 7$, df = 3, P = 0.0719; Reader 2: CV = 4.5%, test of symmetry: $\chi^2 = 7$, df = 4, P = 0.1359).

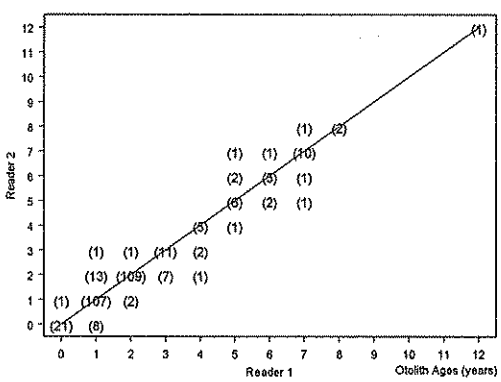


Figure 2. Between-reader comparison of otolith age estimates for bluefish in 2006.

Of the 323 fish aged with otoliths 10 age classes were represented (Table 1). The average age for the sample was 2.1 years, and the standard deviation and standard error were 1.78 and 0.10, respectively.

Year-class data (Figure 3) indicates that recruitment into the fishery began at age 0, which corresponded to the 2006 year-class for bluefish caught in 2006. One and two-year-old fish were the dominant year-classes in the 2006 sample.

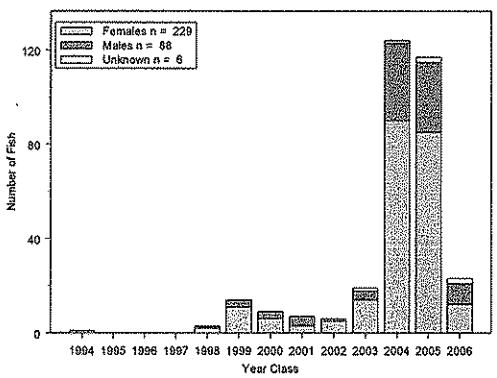


Figure 3. Year-class distribution for bluefish collected for ageing in 2006. Distribution is broken down by sex.

Age-Length-Key — In Table 2 we present an age-length-key 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. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

S-Plus. 1999. S-Plus 4.5 Guide to Statistics. Data Analysis Products Division. Math Soft, Inc. Seattle, Washington.

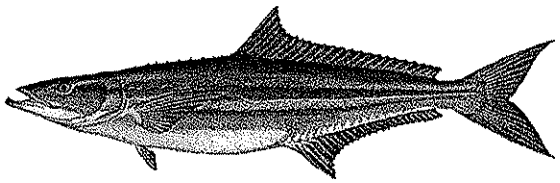
Table 1. The number of bluefish assigned to each total length-at-age category for 323 fish collected for age determination in Virginia in 2006.

Length 1-inch intervals	Age (years)										
	0	1	2	3	4	5	6	7	8	12	Totals
8 - 8.99	2	0	0	0	0	0	0	0	0	0	2
9 - 9.99	2	0	0	0	0	0	0	0	0	0	2
10 - 10.99	4	1	0	0	0	0	0	0	0	0	5
11 - 11.9	7	1	0	0	0	0	0	0	0	0	8
12 - 12.99	4	10	1	0	0	0	0	0	0	0	15
13 - 13.99	2	15	0	0	0	0	0	0	0	0	17
14 - 14.99	1	40	3	0	0	0	0	0	0	0	44
15 - 15.99	1	25	17	0	0	0	0	0	0	0	43
16 - 16.99	0	7	26	1	0	0	0	0	0	0	34
17 - 17.99	0	5	24	5	1	0	0	0	0	0	35
18 - 18.99	0	7	12	2	0	0	0	0	0	0	21
19 - 19.99	0	2	13	3	0	1	0	0	0	0	19
20 - 20.99	0	1	10	1	0	0	0	0	0	0	12
21- 21.99	0	1	7	1	0	0	0	0	0	0	9
22 - 22.99	0	1	4	1	0	0	0	0	0	0	6
23 -23.99	0	0	6	2	1	0	0	0	0	0	9
24 - 24.99	0	0	1	1	0	0	0	0	0	0	2
25 - 25.99	0	1	0	0	0	0	0	0	0	0	1
26 - 26.99	0	0	0	2	0	2	0	0	0	0	4
27 - 27.99	0	0	0	0	1	0	0	0	0	0	1
28 - 28.99	0	0	0	0	1	0	0	0	0	0	1
29 - 29.99	0	0	0	0	1	0	1	0	0	0	2
30 - 30.99	0	0	0	0	0	2	0	1	0	0	3
31 - 31.99	0	0	0	0	1	1	1	2	0	0	5
32 - 32.99	0	0	0	0	0	1	2	4	0	0	7
33 - 33.99	0	0	0	0	0	0	1	2	2	1	6
34 - 34.99	0	0	0	0	0	0	4	5	0	0	9
35 - 35.99	0	0	0	0	0	0	0	0	1	0	1
Totals	23	117	124	19	6	7	9	14	3	1	323

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-class, based on otolith ages, for bluefish collected for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)										
	0	1	2	3	4	5	6	7	8	12	N
8 - 8.99	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
9 - 9.99	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
10 - 10.99	0.800	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5
11 - 11.9	0.875	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8
12 - 12.99	0.267	0.667	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	15
13 - 13.99	0.118	0.882	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	17
14 - 14.99	0.023	0.909	0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000	44
15 - 15.99	0.023	0.581	0.395	0.000	0.000	0.000	0.000	0.000	0.000	0.000	43
16 - 16.99	0.000	0.206	0.765	0.029	0.000	0.000	0.000	0.000	0.000	0.000	34
17 - 17.99	0.000	0.143	0.686	0.143	0.029	0.000	0.000	0.000	0.000	0.000	35
18 - 18.99	0.000	0.333	0.571	0.095	0.000	0.000	0.000	0.000	0.000	0.000	21
19 - 19.99	0.000	0.105	0.684	0.158	0.000	0.053	0.000	0.000	0.000	0.000	19
20 - 20.99	0.000	0.083	0.833	0.083	0.000	0.000	0.000	0.000	0.000	0.000	12
21- 21.99	0.000	0.111	0.778	0.111	0.000	0.000	0.000	0.000	0.000	0.000	9
22 - 22.99	0.000	0.167	0.667	0.167	0.000	0.000	0.000	0.000	0.000	0.000	6
23 -23.99	0.000	0.000	0.667	0.222	0.111	0.000	0.000	0.000	0.000	0.000	9
24 - 24.99	0.000	0.000	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	2
25 - 25.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
26 - 26.99	0.000	0.000	0.000	0.500	0.000	0.500	0.000	0.000	0.000	0.000	4
27 - 27.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1
28 - 28.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1
29 - 29.99	0.000	0.000	0.000	0.000	0.500	0.000	0.500	0.000	0.000	0.000	2
30 - 30.99	0.000	0.000	0.000	0.000	0.000	0.667	0.000	0.333	0.000	0.000	3
31 - 31.99	0.000	0.000	0.000	0.000	0.200	0.200	0.200	0.400	0.000	0.000	5
32 - 32.99	0.000	0.000	0.000	0.000	0.000	0.143	0.286	0.571	0.000	0.000	7
33 - 33.99	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.333	0.333	0.167	6
34 - 34.99	0.000	0.000	0.000	0.000	0.000	0.000	0.444	0.556	0.000	0.000	9
35 - 35.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1
									Sample size		323

Chapter 4
Cobia



Rachycentron canadum

INTRODUCTION

A total of 29 cobia, *Rachycentron canadum*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. The average age of the sample was 6.3 years, and the standard deviation and standard error were 3.32 and 0.62, respectively. Eleven age classes (3 to 9, 11 to 12, 14, and 16) were represented, comprising fish from the 1990, 1992, 1994 to 1995, 1997 to 2003 year-classes. The 2002 year-class dominated the sample.

METHODS

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 in labeled cell well plates.

Preparation — Individual otoliths were placed into 14 mm x 5 mm x 3 mm wells (Ladd Industries silicon rubber mold) filled with Loctite adhesive. Each otolith was rolled around in the Loctite to remove all trapped air bubbles and ensure complete coverage of the otolith surface. The otoliths were oriented sulcal side down with the long axis of the otolith exactly parallel with the long axis of the mold well. 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 Crystal Bond onto a standard microscope slide. Once mounted, a small mark was placed on the otolith surface directly above the otolith focus. The slide, with attached otolith, was then secured to an Isomet saw equipped with two diamond wafering blades separated by a 0.5 mm spacer, which was slightly smaller in diameter than the diamond blades. The otolith was positioned so that the wafering 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 wafer section was viewed under a dissecting microscope to determine which side (cut surface) of the otolith was closer to the focus. The otolith section was mounted best-side up onto a glass slide with Crystal Bond. The section was then lightly polished on a Buehler Ecomet 3 variable speed grinder-polisher with Mark V Laboratory 30-micron polishing film. After drying, a thin layer of Flo-texx mounting medium was applied over the polished otolith surface, which provided enhanced contrast and greater readability by increasing light transmission through the sections.

Readings — Two different readers using a LEICA MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification

aged all sectioned otoliths (Figure 1). Both age readers aged all of the otolith samples.

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

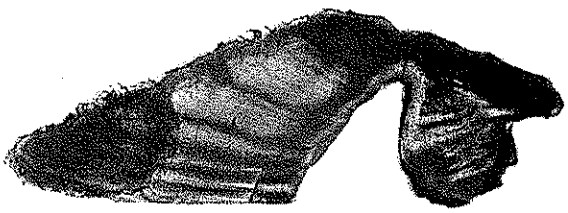


Figure 1. Otolith thin-section from a 1524mm TL 6 year old cobia.

Comparison Tests — Reader 1 aged all fish for a second time to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was very high for Reader 1 with the CV of 0.2%). There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 6$, df = 5, $P = 0.3062$). Figure 2 illustrates the between readers' precision of age estimates. The average coefficient of variation (CV) of 2.5% was not significant.

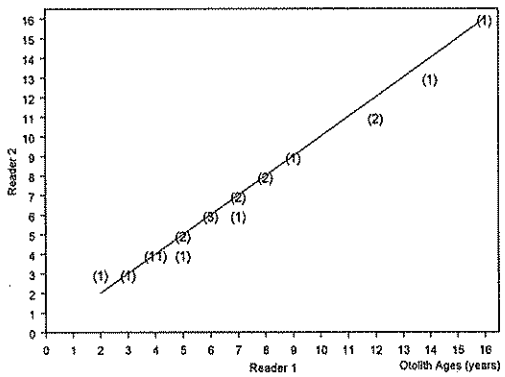


Figure 2. Between-reader

There was no evidence of drift in age determination from Year 2000 precision fish for Reader 1. Agreement for Reader 1 was 84% with a CV of 1.4% (test of symmetry: $\chi^2 = 8$, df = 5, P = 0.1562). There was evidence of drift in age determination from Year 2000 precision fish for Reader 2. Agreement for Reader 2 was 60% with a CV of 4.7% (test of symmetry: $\chi^2 = 20$, df = 10, P = 0.0293). Reader 2 over-aged 30% of Year 2000 precision fish. Following our ageing policies, both Reader 1 and Reader 2 will retrieve and examine the otoliths of the over-aged fish to identify potential causes of the overestimation before we start to age next year.

Of the 29 fish aged, 11 age classes were represented (Table 1). The average age of

the sample was 6.3 years, and the standard deviation and standard error were 3.32 and 0.62, respectively.

Year-class data (Figure 3) indicates that recruitment into the fishery begins at age 3, which corresponds to the 2003 year-class for cobia caught in 2006. The year-class 2002 dominated the sample.

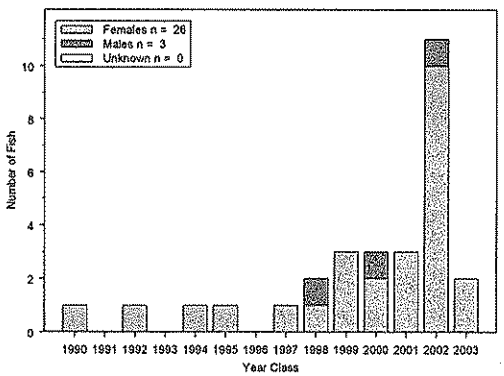


Figure 3. Year-class distribution for cobia collected for ageing in 2006. Distribution is broken down by sex.

Age-Length-Key — In Table 2 we present an age-length-key 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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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.

S-Plus. 1999. S-Plus 4.5 Guide to Statistics. Data Analysis Products Division. Math Soft, Inc. Seattle, Washington.

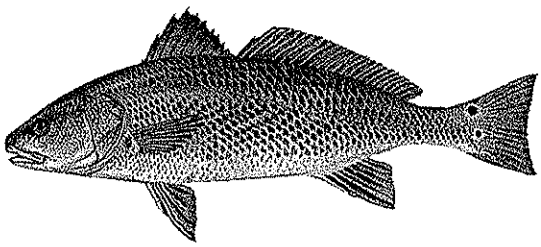
Table 1. The number of cobia assigned to each total length-at-age category for 28 fish sampled for age determination in Virginia during 2006 (No length available for 1 fish).

Length 1-inch intervals	Age (years)											
	3	4	5	6	7	8	9	11	12	14	16	Total
37 - 37.99	1	0	0	0	0	0	0	0	0	0	0	1
39 - 39.99	0	1	0	0	0	0	0	0	0	0	0	1
41 - 41.99	0	1	0	0	0	1	0	0	0	0	0	2
45 - 45.99	0	2	0	0	0	0	0	0	0	0	0	2
46 - 46.99	1	1	0	0	0	0	0	0	0	0	0	2
48 - 48.99	0	3	0	0	0	0	0	0	0	0	0	3
49 - 49.99	0	1	0	0	0	0	0	0	0	0	0	1
50 - 50.99	0	1	0	0	0	0	0	0	0	0	0	1
51 - 51.99	0	0	0	1	0	1	0	0	0	0	0	2
53 - 53.99	0	0	2	0	0	0	0	0	0	0	0	2
54 - 54.99	0	0	1	1	1	0	0	0	0	0	0	3
55 - 55.99	0	0	0	0	1	0	0	0	0	0	0	1
58 - 58.99	0	0	0	1	0	0	0	0	0	0	0	1
59 - 59.99	0	0	0	0	1	0	0	0	0	0	0	1
60 - 60.99	0	0	0	0	0	0	0	0	0	0	1	1
61 - 61.99	0	0	0	0	0	0	1	0	0	0	0	1
64 - 64.99	0	0	0	0	0	0	0	1	0	0	0	1
65 - 65.99	0	0	0	0	0	0	0	0	1	0	0	1
68 - 68.99	0	0	0	0	0	0	0	0	0	1	0	1
Total	2	10	3	3	3	2	1	1	1	1	1	28

Table 2. Age-Length key, as proportions-at-age in each 1 in length-interval, based on otolith ages for cobia sampled for age determination in Virginia during 2006 (No length available for 1 fish).

Length 1-inch intervals	Age (years)											
	3	4	5	6	7	8	9	11	12	14	16	N
37 - 37.99	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
39 - 39.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
41 - 41.99	0.000	0.500	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	2
45 - 45.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
46 - 46.99	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
48 - 48.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3
49 - 49.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
50 - 50.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
51 - 51.99	0.000	0.000	0.000	0.500	0.000	0.500	0.000	0.000	0.000	0.000	0.000	2
53 - 53.99	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
54 - 54.99	0.000	0.000	0.333	0.333	0.333	0.000	0.000	0.000	0.000	0.000	0.000	3
55 - 55.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1
58 - 58.99	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
59 - 59.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1
60 - 60.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1
61 - 61.99	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1
64 - 64.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1
65 - 65.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1
68 - 68.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1
										Sample Size		28

Chapter 5
Red Drum



Sciaenops ocellatus

INTRODUCTION

A total of 16 red drum, *Sciaenops ocellatus*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. The average age of the sample was 1.6 years, and the standard deviation and standard error were 0.81 and 0.20, respectively. Three age classes (1, 2 and 3) were represented, comprising fish from the 2003, 2004, and 2005 year-classes. One-year-old fish were the dominant year-class in the 2005 sample.

METHODS

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 in their original labeled coin envelopes.

Preparation — Otoliths were processed for ageing following the methods described in Bobko (1991) for black drum. Briefly, otoliths were mounted on glass slides with Crystal Bond. At least two serial transverse sections were cut through the nucleus of each otolith with a Buehler Isomet low-speed saw equipped with a three inch, fine grit Norton diamond-wafering blade. After drying, a thin layer of Flo-texx mounting medium was applied to the otolith section to increase light transmission through the translucent zones, which provided enhanced contrast and greater readability.

Readings — Two different readers aged all sectioned otoliths using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 1).

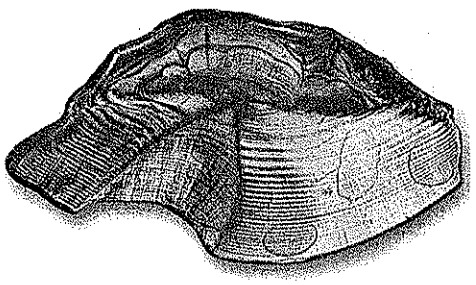


Figure 1. Otolith thin-section from 26 year old red drum.

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 ages were based on a biological birthdate of September 1, while year-class assignment was based on a January 1 annual birthdate. Red drum were treated in this manner because of the timing of spawning and the fact that the first annulus is not seen on an otolith until a fish's second spring. For example, a red drum that was born in September of 1997 and captured in March of 1999 would not have any visible annuli on its otoliths, but would be aged as a 1 year-old fish since it lived beyond one September (September 1998). But this 1 year-old fish caught in 1999 would be mistakenly assigned to the 1998 year-class. In order to properly assign the fish to its correct year-class, 1997, a January birthdate was used which would make the fish 2 years-old (since the fish lived past January 1998 and 1999) and year-class would be assigned correctly.

Comparison Tests — Reader 1 aged all fish for second time to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Measurements of reader self-precision were very high, with Reader 1 able to reproduce 100 % of the ages of previously read otoliths. Figure 2 illustrates the between readers' precision of age estimates. There was 100% agreement between readers. There was no evidence of drift in age determination from Year 2000 precision fish. Agreement was 100% for Reader 1 and 90% for Reader 2 (CV = 3.2%, test of symmetry: $\chi^2 = 2$, df = 2, P = 0.3679), respectively.

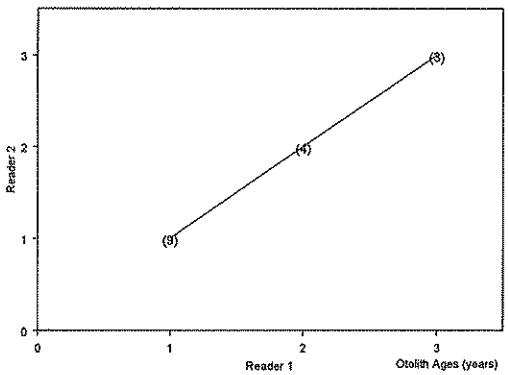


Figure 2. Between-reader comparison of otolith age estimates for red drum in 2006

Of the 16 fish aged with otoliths, 3 age classes were represented (Table 1). The average age of the sample was 1.6 years, and the standard deviation and standard error were 0.81 and 0.20, respectively.

Year-class data (Figure 3) indicate that the 2005 year-class dominated the sample. Indicative of the trend in the recreational fishing, very few older fish were collected in 2006.

Age-Length-Key — In Table 2 we present an age-length-key that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

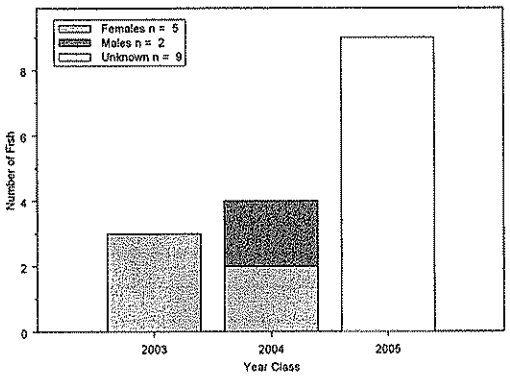


Figure 3. Year-class distribution for red drum collected for ageing in 2006. Distribution is broken down by sex.

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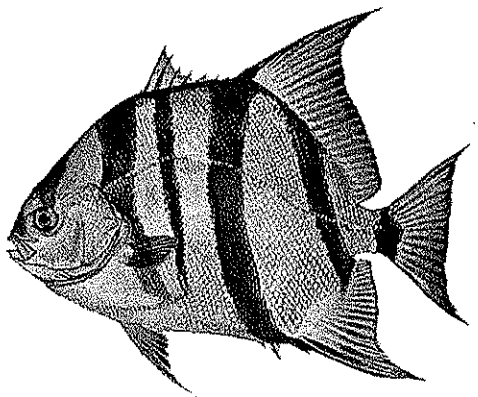
Table 1. The number of red drum assigned to each total length-at-age category for 16 fish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)			
	1	2	3	Total
11 - 11.99	1	0	0	1
12 - 12.99	2	0	0	2
13 - 13.99	2	0	0	2
18 - 18.99	4	0	0	4
23 - 23.99	0	2	0	2
24 - 24.99	0	1	0	1
26 - 26.99	0	1	0	1
27 - 27.99	0	0	2	2
28 - 28.99	0	0	1	1
Total	9	4	3	16

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-intervals, based on otolith ages for red drum sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)			
	1	2	3	N
11 - 11.99	1.000	0.000	0.000	1
12 - 12.99	1.000	0.000	0.000	2
13 - 13.99	1.000	0.000	0.000	2
18 - 18.99	1.000	0.000	0.000	4
23 - 23.99	0.000	1.000	0.000	2
24 - 24.99	0.000	1.000	0.000	1
26 - 26.99	0.000	1.000	0.000	1
27 - 27.99	0.000	0.000	1.000	2
28 - 28.99	0.000	0.000	1.000	1
			Sample Size	16

Chapter 6
Atlantic Spadefish



Chaetodipterus faber

INTRODUCTION

A total of 335 spadefish, *Chaetodipterus faber*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. We were unable to age 9 fish due to the damage of their otoliths. The average age of the sample was 3.1 years, and the standard deviation and standard error were 2.45 and 0.14, respectively. Fifteen age classes (0 to 12, 14, 19) were represented, comprising fish from the 1987, 1992, 1994 to 2006 year-classes.

METHODS

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 in labeled cell well trays.

Preparation — Otoliths were processed for ageing using a bake and thin-section technique. Preparation began by randomly selecting either the right or left otolith. The otolith was mounted with Crystal Bond onto a standard microscope slide with its distal surface orientated upwards. Once mounted, a small mark was placed on the otolith surface directly above the otolith focus. The slide, with attached otolith, was then secured to a Buehler Isomet low-speed saw equipped with two fine grit Norton diamond-wafering blades separated by a 0.5 mm spacer, which was slightly smaller in diameter than the diamond blades. The otolith was positioned so that the wafering blades straddled each side of the otolith focus ink 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 section was placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400°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 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.

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

Readings — Two different readers aged all sectioned otoliths using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 1).

All samples were aged in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2003. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2003 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Measurements of reader self-precision were low (Reader 1's CV = 8.4% and Reader 2's CV = 11.9%). Figure 2 illustrates the between readers' precision of age estimates. There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 18.4$, df = 14, $P = 0.1906$). The average coefficient of variation (CV) of 3.0% was considered not to be significant and lower than the CV of 5.3% in 2005. There was no evidence of drift in age determination from Year 2003 precision fish. Agreement was 72% for Reader 1 (CV = 5.4%, test of symmetry: $\chi^2 = 8.7$, df = 9, P = 0.4686) and 84% for Reader 2 (CV = 1.9%, test of symmetry: $\chi^2 = 6$, df = 7, P = 0.5398).

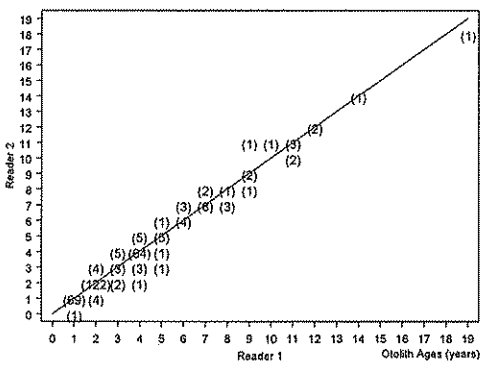


Figure 2. Between-reader comparison of otolith age estimates for spadefish in 2006.

Of the 326 fish aged with otoliths, 15 age classes were represented (Table 1). The average age of the sample was 3.1 years, and the standard deviation and standard error were 2.45 and 0.14, respectively. Year-class data (Figure 3) indicate that the 2002, 2004, and 2005 year-classes dominated the sample.

Age-Length-Key — In Table 2 we present an age-length-key that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

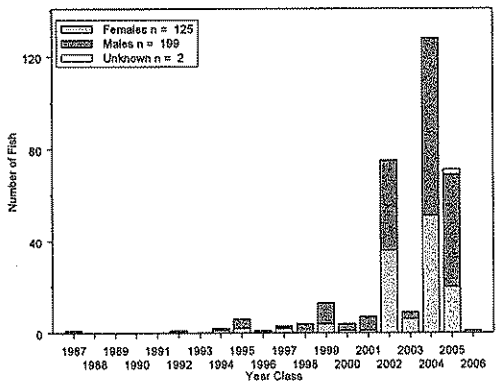


Figure 3. Year-class distribution for spadefish collected for ageing in 2006. Distribution is broken down by sex.

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

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Table 1. The number of spadefish assigned to each total length-at-age category for 326 fish collected for age determination in Virginia during 2006.

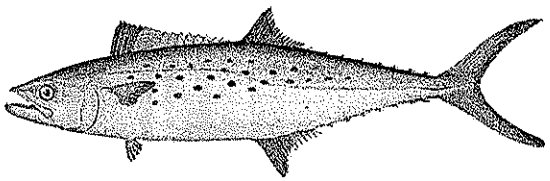
Length 1-inch intervals	Age (years)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	14	19	Total
4 - 4.99	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
5 - 5.99	0	32	2	0	0	0	0	0	0	0	0	0	0	0	0	34
6 - 6.99	0	30	23	0	0	0	0	0	0	0	0	0	0	0	0	53
7 - 7.99	1	6	46	1	1	0	0	0	0	0	0	0	0	0	0	55
8 - 8.99	0	0	23	1	1	0	0	0	0	0	0	0	0	0	0	25
9 - 9.99	0	0	24	2	1	0	0	0	0	0	0	0	0	0	0	27
10 - 10.99	0	0	5	0	3	0	0	0	0	0	0	0	0	0	0	8
11 - 11.99	0	0	5	0	7	0	0	0	0	0	0	0	0	0	0	12
12 - 12.99	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	11
13 - 13.99	0	0	0	0	16	1	0	0	0	0	0	0	0	0	0	17
14 - 14.99	0	0	0	0	11	1	0	0	0	0	0	0	0	0	0	12
15 - 15.99	0	0	0	2	15	0	0	0	0	0	0	0	0	0	0	17
16 - 16.99	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	6
17 - 17.99	0	0	0	2	4	2	3	3	0	0	0	0	0	0	0	14
18 - 18.99	0	0	0	0	0	3	1	3	1	0	0	0	0	0	0	8
19 - 19.99	0	0	0	0	0	0	0	2	1	1	0	2	0	0	0	6
20 - 20.99	0	0	0	0	0	0	0	2	1	0	1	1	1	0	0	6
21 - 21.99	0	0	0	0	0	0	0	2	1	2	0	2	0	0	1	8
22 - 22.99	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2
23 - 23.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Total	1	71	128	9	75	7	4	13	4	3	1	6	2	1	1	326

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-intervals, based on otolith ages for spadefish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	14	19	N
4 - 4.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3
5 - 5.99	0.000	0.941	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	34
6 - 6.99	0.000	0.566	0.434	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	53
7 - 7.99	0.018	0.109	0.836	0.018	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	55
8 - 8.99	0.000	0.000	0.920	0.040	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	25
9 - 9.99	0.000	0.000	0.889	0.074	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	27
10 - 10.99	0.000	0.000	0.625	0.000	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8
11 - 11.99	0.000	0.000	0.417	0.000	0.583	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	12
12 - 12.99	0.000	0.000	0.000	0.091	0.909	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	11
13 - 13.99	0.000	0.000	0.000	0.000	0.941	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	17
14 - 14.99	0.000	0.000	0.000	0.000	0.917	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	12
15 - 15.99	0.000	0.000	0.000	0.118	0.882	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	17
16 - 16.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6
17 - 17.99	0.000	0.000	0.000	0.143	0.286	0.143	0.214	0.214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	14
18 - 18.99	0.000	0.000	0.000	0.000	0.000	0.375	0.125	0.375	0.125	0.000	0.000	0.000	0.000	0.000	0.000	8
19 - 19.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.167	0.167	0.000	0.333	0.000	0.000	0.000	6
20 - 20.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.167	0.000	0.167	0.167	0.167	0.000	0.000	6
21 - 21.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.125	0.250	0.000	0.250	0.000	0.000	0.125	8
22 - 22.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.500	0.000	2
23 - 23.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1
24 - 24.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1
														Sample Size		326

Chapter 7

Spanish Mackerel



Scomberomorous maculatus

INTRODUCTION

A total of 418 Spanish mackerel, *Scomberomorous maculatus*, was collected by the Virginia Marine Resource Commission (VMRC) Biological Sampling Program in 2006. We selected 291 fish for age and growth analysis (Please see Chapter 14). The average age was 1.8 years, and the standard deviation and standard error were 1.12 and 0.07, respectively. Seven age classes were observed (1 to 6, and 8), representing fish from the 1998, 2000 through 2005 year-classes.

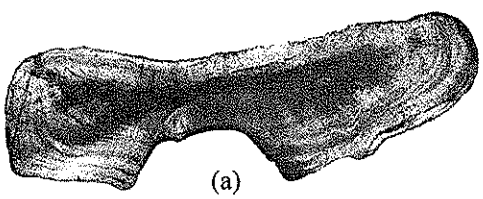
METHODS

Handling of collection — All 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 sample number. All otoliths were stored dry in labeled cell well plates.

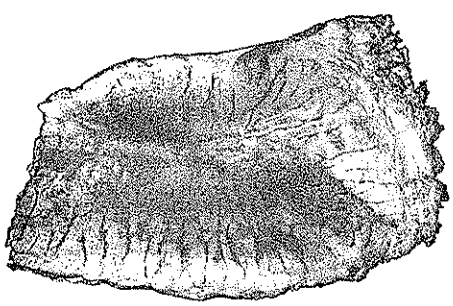
Preparation — Otoliths from fish were processed using an Age and Growth Laboratory thin section technique modified to deal with the fragile nature of Spanish mackerel otoliths. Briefly, an otolith was first embedded in a 9.5 mm x 4.5 mm x 4.5 mm silicon mold well with Loctite 349 photo-active adhesive. The mold was placed under ultraviolet light to cure and harden the Loctite. The embedded otolith was removed from the Silicon mold and the location of the core of the otolith was then marked with an extra fine point permanent marker. A thin transverse section was made using a Buelher Isomet saw equipped with two high concentration Norton diamond wafering blades separated by a 0.4 mm steel spacer. The otolith section was mounted best-side up onto a glass slide with Crystal Bond. The section was then lightly polished on a Buehler Ecomet 3 variable speed grinder-polisher with Mark V Laboratory 30-micron polishing film. The thin-section was then 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 — By convention, a birth date of January 1 is assigned to all Northern Hemisphere fish species. We use a system of age determination that assigns age class according to the date of sacrifice with respect to this international accepted birth date and the timing of annulus formation. Although an otolith annulus is actually the combination of an opaque and translucent band, when ageing otoliths we actually enumerate only the opaque bands, but still refer to them as annuli. Spanish mackerel otolith annulus

formation occurs between the months of April and June, with younger fish tending to lay down annuli earlier than older fish. Fish age is written first followed by the actual number of annuli visible listed within parentheses (e.g., 3(3)). The presence of a "+" after the number in the parentheses indicates new growth, or "plus growth" visible on the structure's margin. Using this method, a fish sacrificed in January before annulus formation with three visible annuli would be assigned the same age, 4(3+), as a fish with four visible annuli sacrificed in August after annulus formation, 4(4+). Year-class is then assigned once the reader determines the fish's age and takes into account the year of capture.



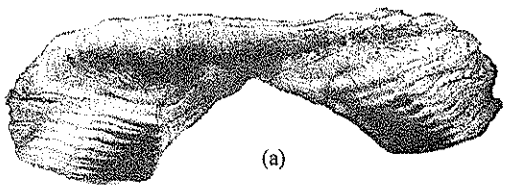
(a)



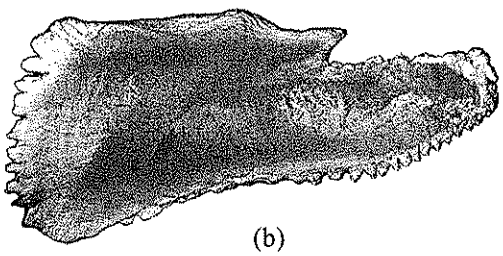
(b)

Figure 1. A three year old spanish mackerel otolith from a 0.6 kg male a) thin-section b) whole otolith with part of the tip broken off.

Two different readers aged all sectioned otoliths using a Leica MZ-12 dissecting microscope with polarized transmitted light at between 8 and 40 times magnification. The first annulus on sectioned otoliths 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 (Figures 1 and 2).



(a)



(b)

Figure 2. An eight year old Spanish mackerel otolith from a 1 kg female a) thin-section b) whole 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.

Comparison Tests — A random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using

the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2003. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2003 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was fair (Reader 1's CV = 11.4% and Reader 2's CV = 8.1%). The average between-reader coefficient of variation (CV) of 11.4% was considered high. Figure 3 illustrates the between readers' precision of age estimates. There was evidence of systematic disagreement between reader 1 and reader 2 (test of symmetry: $\chi^2 = 46.1$, df = 7, $P < 0.0001$). The between-reader agreement for otoliths for one year or less was 100% of all aged fish. The high agreement and the high CV were partially due to the sample dominated by younger fish.

There was evidence of small drift in age determination from Year 2003 precision fish for Reader 1. Agreement for Reader 1 was 82% with a CV of 7.2% (test of symmetry: $\chi^2 = 9$, df = 2, P = 0.0111). Reader 1 over-aged 18% of the precision fish. There was no evidence of drift in age determination from Year 2003 precision fish for Reader 2. Agreement for Reader 2 was 92% with a CV of 2.3% (test of symmetry: $\chi^2 = 4$, df = 3, P = 0.2615). Following our ageing policies, both Reader 1 and Reader 2 will retrieve and examine the otoliths of the over-aged fish to identify potential causes of the overestimation before we start to age next year.

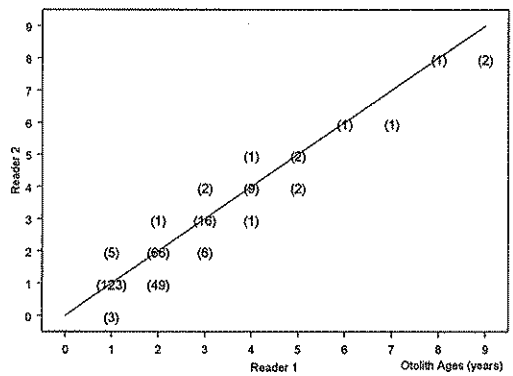


Figure 3. Between-reader comparison of otolith age estimates for Spanish mackerel in 2006.

Of the 291 Spanish mackerel aged with otoliths, 7 age classes were represented (Table 3). The average age was 1.8 year old, and the standard deviation and standard error were 1.12 and 0.07, respectively. Year-class data (Figure 4)

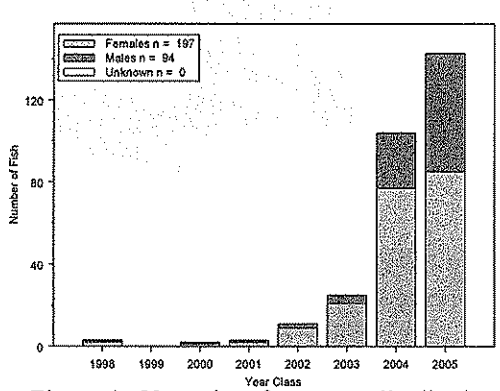


Figure 4. Year-class frequency distribution for Spanish mackerel collected for ageing in 2006. Distribution for otolith ages is broken down by sex.

show that the fishery was comprised of 7 year-classes, comprising fish from the 1998, 2000 through 2005 year-classes, with fish primarily from the 2004 and 2005 year-classes.

Age-Length-Key — In Table 2 we present an age-length-key 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 etermining the consistency of age terminations. Trans. Am. Fish. Soc. 124:131-138.

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Kimura, D.K. 1980. Likelihood methods for the von Bertalanffy growth curve. Fish. Bull. 77:765-776.

Murphy E.O., R.S. Birdsong, J.A. Musick. 1997. Fishes of the Chesapeake Bay. Smithsonian Institute Press. Washington and London.

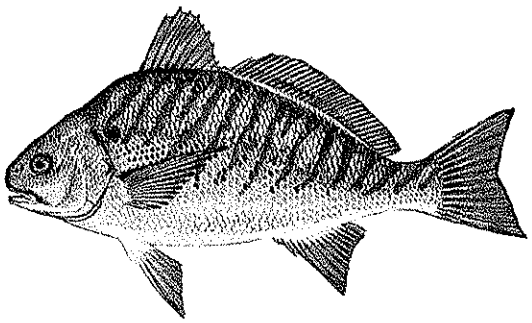
Table 1. The number of Spanish mackerel assigned to each total length-at-age category for 291 fish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)							
	1	2	3	4	5	6	8	Total
11 - 11.99	0	1	0	0	0	0	0	1
13 - 13.99	1	2	0	0	0	0	0	3
14 - 14.99	12	6	0	0	0	0	0	18
15 - 15.99	43	17	0	0	0	0	0	60
16 - 16.99	43	20	1	0	0	0	0	64
17 - 17.99	32	15	3	0	0	0	0	50
18 - 18.99	10	12	1	0	0	0	0	23
19 - 19.99	1	6	5	0	1	0	0	13
20 - 20.99	1	5	2	1	0	0	0	9
21 - 21.99	0	10	7	1	0	1	0	19
22 - 22.99	0	7	4	4	0	0	0	15
23 - 23.99	0	3	0	1	1	1	0	6
24 - 24.99	0	0	2	3	0	0	0	5
25 - 25.99	0	0	0	0	1	0	0	1
26 - 26.99	0	0	0	1	0	0	0	1
27 - 27.99	0	0	0	0	0	0	2	2
30 - 30.99	0	0	0	0	0	0	1	1
Total	143	104	25	11	3	2	3	291

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-intervals, based on otolith ages for Spanish mackerel sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)							
	1	2	3	4	5	6	8	N
11 - 11.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1
13 - 13.99	0.333	0.667	0.000	0.000	0.000	0.000	0.000	3
14 - 14.99	0.667	0.333	0.000	0.000	0.000	0.000	0.000	18
15 - 15.99	0.717	0.283	0.000	0.000	0.000	0.000	0.000	60
16 - 16.99	0.672	0.313	0.016	0.000	0.000	0.000	0.000	64
17 - 17.99	0.640	0.300	0.060	0.000	0.000	0.000	0.000	50
18 - 18.99	0.435	0.522	0.043	0.000	0.000	0.000	0.000	23
19 - 19.99	0.077	0.462	0.385	0.000	0.077	0.000	0.000	13
20 - 20.99	0.111	0.556	0.222	0.111	0.000	0.000	0.000	9
21 - 21.99	0.000	0.526	0.368	0.053	0.000	0.053	0.000	19
22 - 22.99	0.000	0.467	0.267	0.267	0.000	0.000	0.000	15
23 - 23.99	0.000	0.500	0.000	0.167	0.167	0.167	0.000	6
24 - 24.99	0.000	0.000	0.400	0.600	0.000	0.000	0.000	5
25 - 25.99	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1
26 - 26.99	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1
27 - 27.99	0.000	0.000	0.000	0.000	0.000	0.000	1.000	2
30 - 30.99	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1
						Sample size		291

Chapter 8
Spot



Leiostomus xanthurus

INTRODUCTION

A total of 384 spot, *Leiostomus xanthurus*, was collected by the VMRC's Biological Sampling Program in 2006. We selected 263 fish for age and growth analysis (Please see Chapter 14). The average age for the sample was 1.8 year old, and the standard deviation and standard error were 1.15 and 0.07, respectively. Five age classes (0 to 4) were represented, comprising fish from the 2002 through 2006 year-classes, with fish predominantly from the 2005 year-class.

METHODS

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 in labeled cell well trays.

Preparation — Otoliths were processed for ageing using a thin-sectioning technique. The first step in otolith preparation was to grind down the otolith in a transverse plane to its core using a Hillquist thin section machine's 320-mesh diamond cup wheel. To prevent distortion of the reading surface, the otolith was ground exactly perpendicular to the reading plane. The ground side of the otolith was then placed face down in a drop of Loctite 349 photo-active adhesive on a labeled glass slide and placed under ultraviolet light to allow the adhesive to harden. The Hillquist thin section machine's cup wheel was used again to grind the otolith, embedded in Loctite, to a thickness of 0.3 to 0.5 mm. Finally, a thin layer of Flo-texx mounting medium was applied to the otolith section to increase light transmission through the translucent zones, which provided enhanced contrast and greater readability.

Readings — Two different readers aged all sectioned otoliths using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 1).

All samples were aged in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both

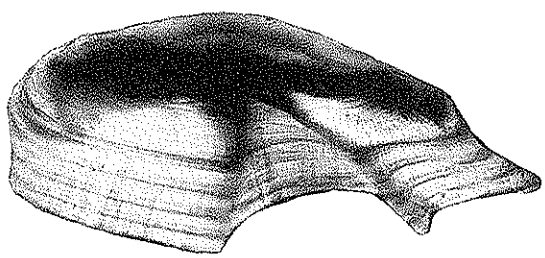


Figure 1. Sectioned otolith from a 5 year old spot.

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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was high for both readers (Reader 1's CV = 2.3% and Reader 2's CV = 1.0%). Measurements of reader precision were high, with age disagreements for only 2 out of 263 fish aged and the average CV of 0.6%. Figure 2 illustrates the between readers' precision of age estimates. There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 2$, df = 2, $P = 0.3679$). There was no evidence of drift in age determination from Year 2000 precision fish with 100% agreement for Read 1 and 98% agreement for Read 2 (CV = 1.0%, test of symmetry: $\chi^2 = 1$, df = 1, P = 0.3173).

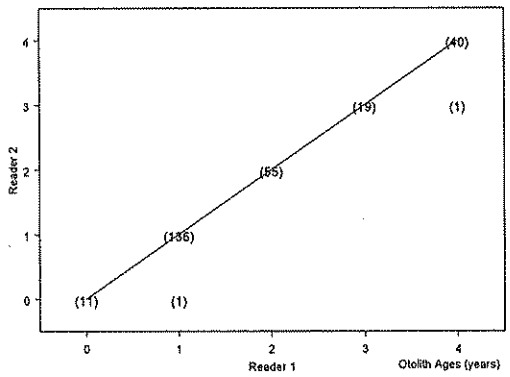


Figure 2. Between-reader comparison of otolith age estimates for spot in 2006.

Of the 263 fish aged with otoliths, 5 age classes were represented (Table 1). The average age for the sample was 1.8 year old, and the standard deviation and standard error were 1.15 and 0.07, respectively.

Year-class data (Figure 3) show that the fishery was comprised of 5 year-classes, with fish spawned in 2005 dominating the catch.

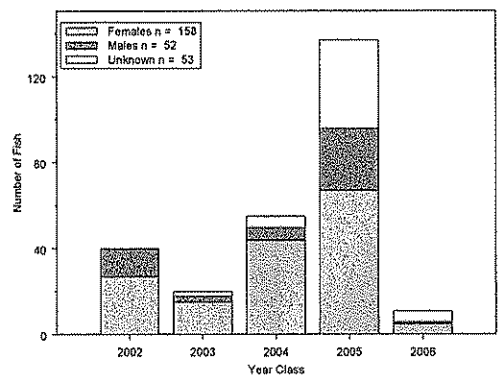


Figure 3. Year-class distribution for spot collected for ageing in 2006. Distribution is broken down by sex.

Age-Length-Key — In Table 2 we present an age-length-key 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. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

S-Plus. 1999. S-Plus 4.5 Guide to Statistics. Data Analysis Products Division. Math Soft, Inc. Seattle, Washington.

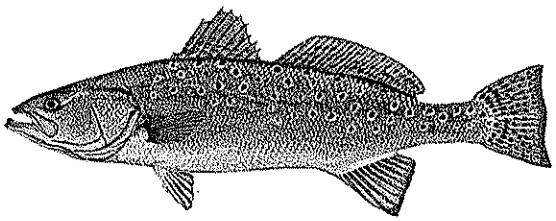
Table 1. The number of spot assigned to each total length-at-age category for 263 fish sampled for age determination in Virginia during 2006.

Length	Age					
1-inch intervals	0	1	2	3	4	Total
6 - 6.99	5	5	0	0	0	10
7 - 7.99	5	17	0	0	0	22
8 - 8.99	1	49	6	1	0	57
9 - 9.99	0	65	36	2	0	103
10 - 10.99	0	1	12	8	17	38
11 - 11.99	0	0	0	4	18	22
12 - 12.99	0	0	1	5	3	9
13 - 13.99	0	0	0	0	2	2
Total	11	137	55	20	40	263

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-interval based on otolith ages for spot sampled for age determination in Virginia during 2006

Length	Age					
1-inch intervals	0	1	2	3	4	N
6 - 6.99	0.500	0.500	0.000	0.000	0.000	10
7 - 7.99	0.227	0.773	0.000	0.000	0.000	22
8 - 8.99	0.018	0.860	0.105	0.018	0.000	57
9 - 9.99	0.000	0.631	0.350	0.019	0.000	103
10 - 10.99	0.000	0.026	0.316	0.211	0.447	38
11 - 11.99	0.000	0.000	0.000	0.182	0.818	22
12 - 12.99	0.000	0.000	0.111	0.556	0.333	9
13 - 13.99	0.000	0.000	0.000	0.000	1.000	2
				Sample size		263

Chapter 9
Spotted Seatrout



Cynoscion nebulosus

INTRODUCTION

A total of 357 spotted seatrout, *Cynoscion nebulosus*, was collected by the VMRC's Biological Sampling Program in 2006. We selected 256 fish (no length available for 1 fish) for age and growth analysis (Please see Chapter 14). The average age for the sample was 1.89 years old, and the standard deviation and standard error were 0.91 and 0.06, respectively. Four age classes (1 to 4) were represented, comprising fish from the 2002 through 2005 year-classes, with fish primarily from the 2004 and 2005 year-classes.

METHODS

Handling of collection — 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 sample number. All otoliths were stored dry in labeled cell well trays.

Preparation — The first step in seatrout otolith preparation was to make a transverse cut just off center of the otolith with a Hillquist thin section machine's cut-off saw equipped with an HCR-100 diamond blade. To prevent distortion of the reading surface, the cut surface of the otolith half containing the focus was ground down on a Hillquist thin section machine's 320 mesh diamond cup wheel until perpendicular to the reading plane. The otolith's ground surface was then placed face down in a drop of Loctite 349 photo-active adhesive on a labeled glass slide and placed under ultraviolet light to allow the adhesive to harden (approximately ten minutes). The Hillquist thin section machine's cup wheel was used again to grind the otolith, embedded in Loctite, to a thickness of 0.3 to 0.5 mm. Finally, a thin layer of Flo-texx mounting medium was applied to the otolith section to increase light transmission through the translucent zones, which provided enhanced contrast and greater readability.

Readings — Two different readers aged all sectioned otoliths using a Leica MZ-12

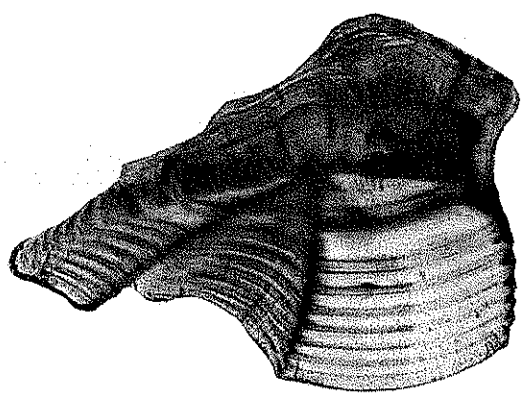


Figure 1. Sectioned otolith from an 8 year old male spotted seatrout.

dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 1). All samples were aged in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was fair for both readers (Reader 1's CV = 2.8% and Reader 2's CV = 2.5%). Measurements of reader precision were high, with age disagreements for only 4 out of 256 fish aged and the average CV of 1.0%. Figure 2 illustrates the between readers' precision of age estimates. There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 2$, df $= 3$, $P = 0.5724$). There was no evidence of drift in age determination from Year 2000 precision fish with 100% agreement for both readers.

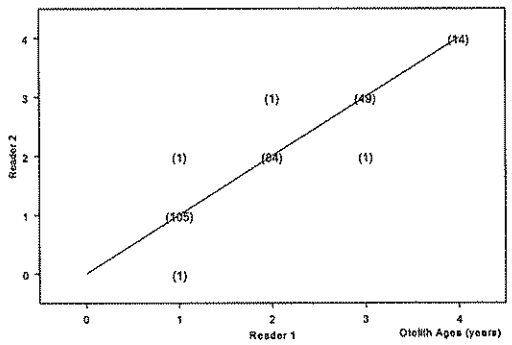


Figure 2. Between-reader comparison of otolith age estimates for spotted seatrout in 2006.

Of the 256 fish aged with otoliths, 4 age classes were represented (Table 1). The average age for the sample was 1.89 years old, and the standard deviation and standard error were 0.91 and 0.06, respectively.

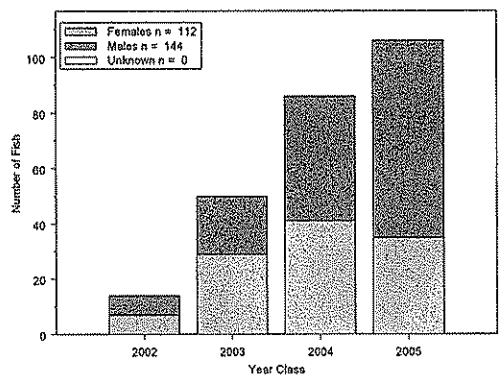


Figure 3. Year-class distribution for spotted seatrout collected for ageing in 2006. Distribution is broken down by sex.

Year-class data (Figure 3) show that the fishery was comprised of 4 year-classes, comprising fish from the 2002-2005 year-classes, with fish primarily from the 2004 and 2005 year-classes.

Age-Length-Key — In Table 2 we present an age-length-key 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. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

S-Plus. 1999. S-Plus 4.5 Guide to Statistics. Data Analysis Products Division. Math Soft, Inc. Seattle, Washington.

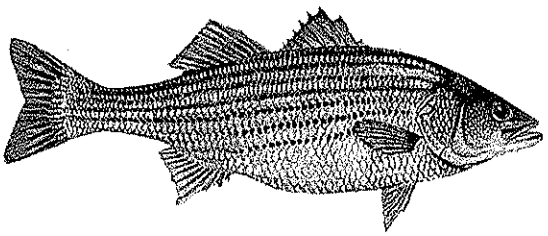
Table 1. The number of spotted seatrout assigned to each total length-at-age category for 256 fish sampled for age determination in Virginia during 2006 (No length available for 1 fish).

Length 1-inch intervals	Age (years)				
	1	2	3	4	Total
9 - 9.99	1	0	0	0	1
10 - 10.99	5	0	0	0	5
11 - 11.99	12	0	0	0	12
12 - 12.99	30	0	0	0	30
13 - 13.99	25	0	0	0	25
14 - 14.99	17	0	0	0	17
15 - 15.99	10	3	0	0	13
16 - 16.99	1	12	0	0	13
17 - 17.99	1	26	0	0	27
18 - 18.99	1	11	1	0	13
19 - 19.99	2	20	2	0	24
20 - 20.99	0	8	10	0	18
21 - 21.99	0	4	8	2	14
22 - 22.99	0	1	5	3	9
23 - 23.99	0	0	11	1	12
24 - 24.99	0	1	9	1	11
25 - 25.99	0	0	4	1	5
26 - 26.99	0	0	0	2	2
27 - 27.99	0	0	0	4	4
Total	105	86	50	14	255

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-intervals, based on otolith ages for spotted seatrout sampled for age determination in Virginia during 2006 (No length available for 1 fish).

Length 1-inch intervals	Age (years)				
	1	2	3	4	N
9 - 9.99	1.000	0.000	0.000	0.000	1
10 - 10.99	1.000	0.000	0.000	0.000	5
11 - 11.99	1.000	0.000	0.000	0.000	12
12 - 12.99	1.000	0.000	0.000	0.000	30
13 - 13.99	1.000	0.000	0.000	0.000	25
14 - 14.99	1.000	0.000	0.000	0.000	17
15 - 15.99	0.769	0.231	0.000	0.000	13
16 - 16.99	0.077	0.923	0.000	0.000	13
17 - 17.99	0.037	0.963	0.000	0.000	27
18 - 18.99	0.077	0.846	0.077	0.000	13
19 - 19.99	0.083	0.833	0.083	0.000	24
20 - 20.99	0.000	0.444	0.556	0.000	18
21 - 21.99	0.000	0.286	0.571	0.143	14
22 - 22.99	0.000	0.111	0.556	0.333	9
23 - 23.99	0.000	0.000	0.917	0.083	12
24 - 24.99	0.000	0.091	0.818	0.091	11
25 - 25.99	0.000	0.000	0.800	0.200	5
26 - 26.99	0.000	0.000	0.000	1.000	2
27 - 27.99	0.000	0.000	0.000	1.000	4
				Sample Size	255

Chapter 10
Striped Bass



Morone saxatilis

INTRODUCTION

A total of 1641 striped bass, *Morone saxatilis*, was collected by the VMRC's Biological Sampling Program in 2006. We selected 913 fish for age and growth analysis (Please see Chapter 14). Of 913 fish aged, 334 fish had both scales and otoliths, 572 fish had scales only, and 7 fish had otoliths only. The average scale age was 9.2 years, with 17 age classes (2 to 17, and 20) comprising fish from the 1986, 1989 to 2004 year-classes. The average otolith age was 8.7 years, with 19 age classes (2 to 19 and 22) comprising fish from the 1984, and 1987 to 2004 year-classes.

METHODS

Handling of collection — 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 sample number. All otoliths were stored dry in labeled cell well plates, while scales were stored in their original coin envelopes.

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 020 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 Crystal Bond onto a standard microscope slide with its distal surface orientated upwards. Once mounted, a small mark was placed on the otolith surface directly above the otolith focus. The slide, with attached otolith, was then secured to an Isomet saw equipped with two diamond wafering blades

separated by a 0.5 mm spacer, which was slightly smaller in diameter than the diamond blades. The otolith was positioned so that the wafering blades straddled each side of the otolith focus ink 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 section was placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400°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 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 — By convention, a birthdate of January 1 is assigned to all Northern Hemisphere fish species. We use a system of age determination that assigns age class according to the date of sacrifice with respect to this international accepted birthdate and the timing of annulus formation, which occurs between the months of May and June for striped bass. Once the reader decides how many annuli are visible on the ageing structure, the year class is assigned. The year class designation, or age, is written first followed by the actual number of annuli visible listed within brackets (e.g. 3(3)). The presence of a "+" after the number in the brackets indicates new growth, or "plus growth" visible on the structure's margin. Using this method, a fish sacrificed in January before annulus formation with three visible annuli would be assigned the same age, 4(3+), as a fish with four visible annuli sacrificed in July after annulus formation, 4(4).

Two different readers aged all samples 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 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.

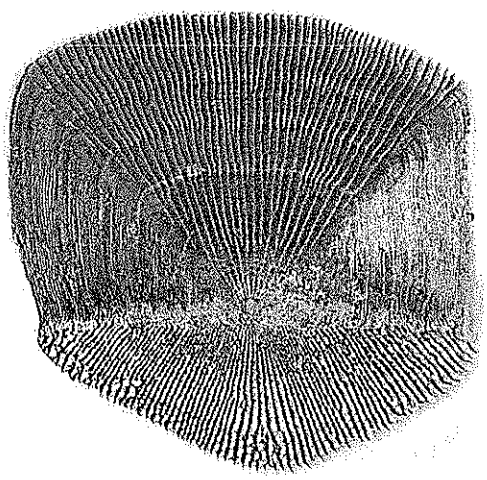


Figure 1. Scale impression of a 5-year-old male striped bass.

Annuli on striped bass scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior\anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli

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

Annuli can also be observed bisecting the perpendicular plain of the radial striations in the anterior field of the scale. Radii emanate out from the focus of the scale towards the outer corner margins of the anterior field. These radial striations consist mainly of segmented concave circuli. The point of intersection between radii and annuli results in a "straightening out" of the concave circuli. This straightening of the circuli should be consistent throughout the entire anterior field of the scale. This event is further amplified by the presence of concave circuli neighboring both directly above and below the annulus.

The first year's annulus can be difficult to locate on some scales. It is typically best identified in the lateral field of the anterior portion of the scale. The distance from the focus to the first year's annulus is typically larger with respect to the following few 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 – Sectioned otoliths were aged by two different readers using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the scales and otoliths of 60 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Scales — Measurements of reader self-precision was marginal for Reader 1 (CV = 6.2%) and good for Reader 2 (CV = 1.8%). In Figure 3 we present a graph of the results for between-reader scale ageing precision. The between-reader agreement for scale for one year or less was 85.2% of all aged fish. The average between-reader coefficient of variation (CV) of 6.1% was marginal. There was evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 82.5$, df = 39, $P < 0.0001$).

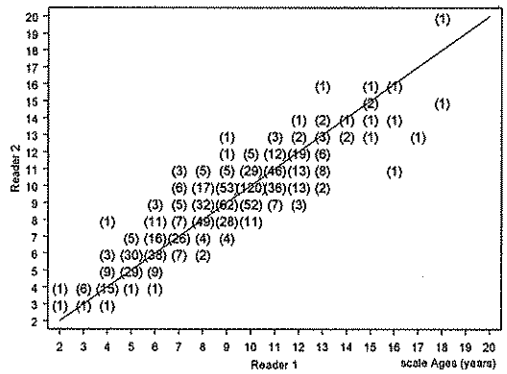


Figure 3. Between-reader comparison of scale age estimates for striped bass in 2006.

Of the 906 striped bass aged with scales, 17 age classes (2 to 17, and 20) were represented. The average age for the sample was 9.2 years. The standard deviation and standard error were 2.41 and 0.08, respectively.

Year-class data (Figure 4) indicates that recruitment into the fishery typically begins at age 2, which corresponds to the 2004 year-class for striped bass caught in 2006. Striped bass appear to fully recruit to the fishery at age 10 (1996 year-class).

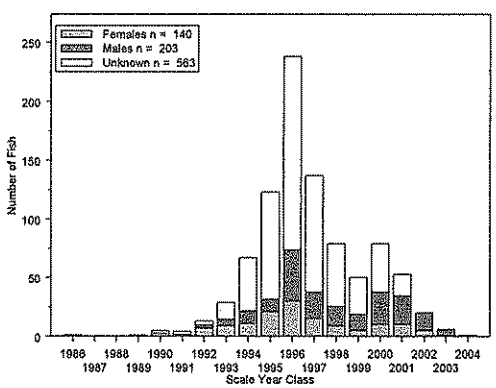


Figure 4. Year-class frequency distribution for striped bass collected for ageing in 2006. Distribution of scale ages is broken down by sex.

There was no evidence of drift in scale age determination from Year 2000 precision fish. Agreement for one year or less was 90% for Reader 1 (CV = 6.0%, test of symmetry: $\chi^2 = 16$, df = 13, P = 0.2491) and 78% for Reader 2 (CV = 8.2%, test of symmetry: $\chi^2 = 18.4$, df = 18, P = 0.4268).

Otoliths — There was good between-reader agreement for otolith age readings using sectioned otoliths, with age differences between the two readers one year or less for 97.4% of all aged fish (Figure 5). The between reader average CV for otolith age estimates was only 2.0%, very comparable to the CV of 2.1% reported for 2005 fish. Unlike scale ages, there was no evidence of systematic disagreement between reader 1 and reader 2 (test of symmetry: $\chi^2 = 23.6$, df = 22, $P = 0.3664$).

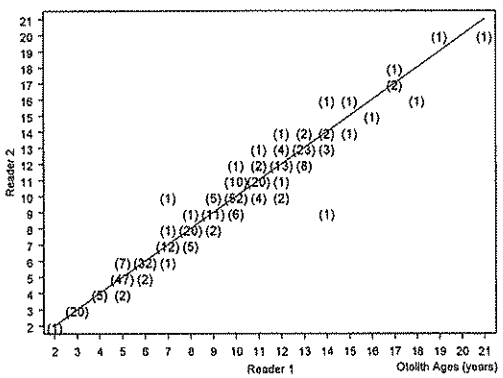


Figure 5. Between-reader comparison of otolith age estimates for striped bass in 2006.

Measurements of reader self-precision were high, with both readers able to reproduce the ages of previously read otoliths (Reader 1's CV = 0.9% and Reader 2's CV = 0.8%).

Of 341 fish aged with otoliths, 19 age classes (2 to 19, and 22) were represented for striped bass aged with otoliths. The average age for the sample was 8.7 years. The standard deviation and standard error were 3.39 and 0.18, respectively.

There was no evidence of drift in otolith age determination from Year 2000 precision fish for Reader 1. Agreement for Reader 1 was 70% (CV = 2.2%, test of symmetry: $\chi^2 = 15$, df = 10, P = 0.1321). There was evidence of drift in age determination from Year 2000 precision fish for Reader 2. Agreement for Reader 2 was 62% (CV = 3.2%, test of symmetry: $\chi^2 = 16.3$, df = 8, P = 0.0379). Reader 2 under-aged 34% of the precision fish. Following our ageing policies, both Reader 1 and Reader 2 will retrieve and examine the otoliths of the under-aged fish to identify potential causes of the underestimation before we start to age next year.

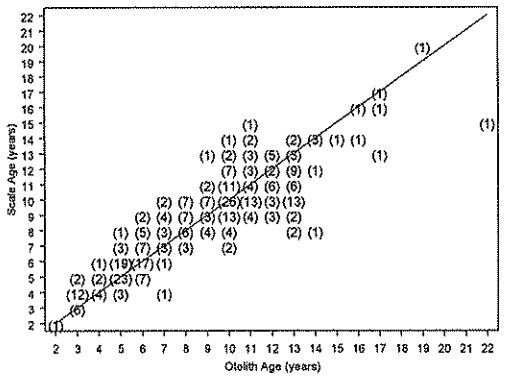


Figure 6. Comparison of otolith and scale age estimates for striped bass in 2006.

Comparison of Scale and Otolith Ages — The CV of otolith and scales age estimates was 9.2%. There was evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 77.7$, df = 39, $P = 0.0002$). Of 334 fish with both scales and otoliths, scales were assigned a lower and higher age than otoliths for 32% and 37% of the fish, respectively (Figure 6).

There was also evidence of bias between otolith and scale ages using an age bias plot (Figure 7), again with scales generally assigned higher ages for younger fish and lower ages for older fish than otoliths age estimates.

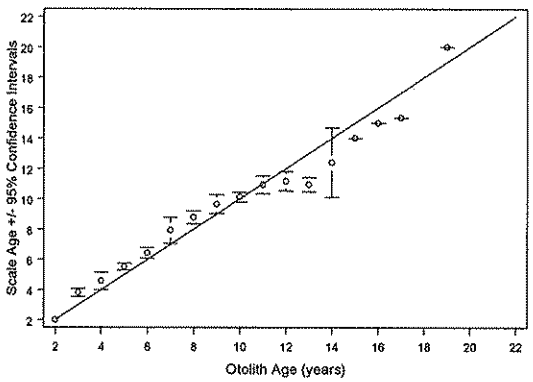


Figure 7. Age-bias plot for striped bass scale and otolith age estimates in 2006.

Age-Length-Key — In Table 2 we present an age-length-key that 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.

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. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

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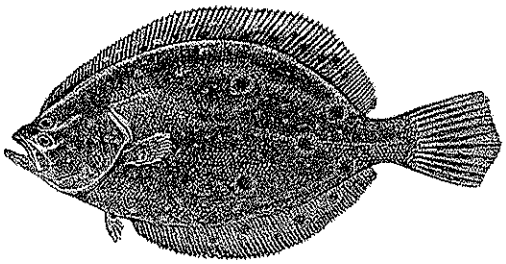
Table 1. The number of striped bass assigned to each total length-at-age category for 906 fish collected for scale-age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)																	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20	Total
15 - 15.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18 - 18.99	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
19 - 19.99	1	2	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	10
20 - 20.99	0	0	4	2	4	1	0	0	0	0	0	0	0	0	0	0	0	11
21 - 21.99	0	0	3	7	2	2	1	0	0	0	0	0	0	0	0	0	0	15
22 - 22.99	0	0	4	7	6	3	1	0	0	0	0	0	0	0	0	0	0	21
23 - 23.99	0	0	0	8	14	7	0	1	3	0	0	0	0	0	0	0	0	33
24 - 24.99	0	0	0	8	13	8	10	2	2	0	1	0	0	0	0	0	0	44
25 - 25.99	0	0	0	6	10	4	12	6	4	0	1	0	0	0	0	0	0	43
26 - 26.99	0	0	0	6	13	4	4	10	12	1	1	0	0	0	0	0	0	51
27 - 27.99	0	0	0	6	5	6	5	10	5	0	1	1	0	0	0	0	0	39
28 - 28.99	0	0	0	0	6	7	10	4	7	3	1	0	0	0	0	0	0	38
29 - 29.99	0	0	0	1	0	5	4	4	4	3	0	1	0	0	0	0	0	22
30 - 30.99	0	0	0	0	1	1	6	3	4	1	1	1	0	0	0	0	0	18
31 - 31.99	0	0	0	0	2	0	2	11	7	1	0	1	0	0	0	0	0	24
32 - 32.99	0	0	0	0	0	1	6	10	8	8	0	2	1	0	0	0	0	36
33 - 33.99	0	0	0	0	1	0	3	12	26	8	4	2	1	0	0	0	0	57
34 - 34.99	0	0	0	0	0	0	9	19	26	14	5	1	0	0	0	0	0	74
35 - 35.99	0	0	0	0	0	1	4	17	41	12	7	2	1	0	0	0	0	85
36 - 36.99	0	0	0	0	0	0	1	17	49	26	11	6	4	0	0	0	0	114
37 - 37.99	0	0	0	0	0	0	1	9	28	17	16	5	1	0	0	0	0	77
38 - 38.99	0	0	0	0	0	0	0	2	5	14	4	0	1	0	0	0	0	26
39 - 39.99	0	0	0	0	0	0	0	0	3	6	7	1	0	1	0	1	0	19
40 - 40.99	0	0	0	0	0	0	0	0	2	5	4	2	1	0	1	0	0	15
41 - 41.99	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	0	0	5
42 - 42.99	0	0	0	0	0	0	0	0	2	1	2	1	3	0	1	0	0	10
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	3
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
53 - 53.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	1	6	20	53	79	50	79	137	238	123	67	29	13	4	5	1	1	906

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-interval, based on scale ages for striped bass sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)																	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20	N
15 - 15.99	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
18 - 18.99	0.000	0.444	0.556	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	9
19 - 19.99	0.100	0.200	0.300	0.200	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10
20 - 20.99	0.000	0.000	0.364	0.182	0.364	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	11
21 - 21.99	0.000	0.000	0.200	0.467	0.133	0.133	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	15
22 - 22.99	0.000	0.000	0.190	0.333	0.286	0.143	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	21
23 - 23.99	0.000	0.000	0.000	0.242	0.424	0.212	0.000	0.030	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	33
24 - 24.99	0.000	0.000	0.000	0.182	0.295	0.182	0.227	0.045	0.045	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	44
25 - 25.99	0.000	0.000	0.000	0.140	0.233	0.093	0.279	0.140	0.093	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	43
26 - 26.99	0.000	0.000	0.000	0.118	0.255	0.078	0.078	0.196	0.235	0.020	0.020	0.000	0.000	0.000	0.000	0.000	0.000	51
27 - 27.99	0.000	0.000	0.000	0.154	0.128	0.154	0.128	0.256	0.128	0.000	0.026	0.026	0.000	0.000	0.000	0.000	0.000	39
28 - 28.99	0.000	0.000	0.000	0.000	0.158	0.184	0.263	0.105	0.184	0.079	0.026	0.000	0.000	0.000	0.000	0.000	0.000	38
29 - 29.99	0.000	0.000	0.000	0.045	0.000	0.227	0.182	0.182	0.182	0.136	0.000	0.045	0.000	0.000	0.000	0.000	0.000	22
30 - 30.99	0.000	0.000	0.000	0.000	0.056	0.056	0.333	0.167	0.222	0.056	0.056	0.056	0.000	0.000	0.000	0.000	0.000	18
31 - 31.99	0.000	0.000	0.000	0.000	0.083	0.000	0.083	0.458	0.292	0.042	0.000	0.042	0.000	0.000	0.000	0.000	0.000	24
32 - 32.99	0.000	0.000	0.000	0.000	0.000	0.028	0.167	0.278	0.222	0.222	0.000	0.056	0.028	0.000	0.000	0.000	0.000	36
33 - 33.99	0.000	0.000	0.000	0.000	0.018	0.000	0.053	0.211	0.456	0.140	0.070	0.035	0.018	0.000	0.000	0.000	0.000	57
34 - 34.99	0.000	0.000	0.000	0.000	0.000	0.000	0.122	0.257	0.351	0.189	0.068	0.014	0.000	0.000	0.000	0.000	0.000	74
35 - 35.99	0.000	0.000	0.000	0.000	0.000	0.012	0.047	0.200	0.482	0.141	0.082	0.024	0.012	0.000	0.000	0.000	0.000	85
36 - 36.99	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.149	0.430	0.228	0.096	0.053	0.035	0.000	0.000	0.000	0.000	114
37 - 37.99	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.117	0.364	0.221	0.208	0.065	0.013	0.000	0.000	0.000	0.000	77
38 - 38.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.192	0.538	0.154	0.000	0.038	0.000	0.000	0.000	0.000	26
39 - 39.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.158	0.316	0.368	0.053	0.000	0.053	0.000	0.053	0.000	19
40 - 40.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.333	0.267	0.133	0.067	0.000	0.067	0.000	0.000	15
41 - 41.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.600	0.000	0.400	0.000	0.000	0.000	0.000	0.000	5
42 - 42.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.100	0.200	0.100	0.300	0.000	0.100	0.000	0.000	10
43 - 43.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	2
44 - 44.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.500	0.000	0.000	2
45 - 45.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.667	0.000	0.000	3
50 - 50.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1
53 - 53.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1
																Sample size		906

Chapter 11
Summer Flounder



Paralichthys dentatus

INTRODUCTION

A total of 1154 summer flounder, *Paralichthys dentatus*, was collected by the VMRC's Biological Sampling Program in 2006. We selected 871 fish for age and growth analysis (Please see Chapter 14). Of 871 fish aged, 496 fish had both scales and otoliths, 330 fish had scales only, and 45 fish had otoliths only. The average scale age was 3.8 years, representing 11 age-classes (1 to 11). Fish from the 2002-2004 year-classes dominated the collection. The average otolith age was 3.8 years, representing 11 year-classes (1 to 11).

METHODS

Handling of collection — 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 sample number. All otoliths were stored dry in labeled cell well plates, while scales were stored in their original coin envelopes.

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 020 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 (Figure 1). The right sagittal otolith was mounted with Aremco's clear Crystal Bond™ 509 adhesive onto a standard microscope slide with its distal surface orientated upwards. Once mounted, a small mark was placed on the otolith surface directly above the otolith focus. The slide, with attached otolith, was then secured to a Buehler Isomet saw equipped with two Norton diamond wafering blades separated by a 0.5 mm stainless steel spacer, which was slightly smaller in diameter than the diamond blades. The otolith was positioned so that the wafering blades straddled each side of the otolith focus ink 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

(a) (b)

Figure 1. Whole otoliths from a 485 mm (total length) female summer flounder. (a) left otolith (b) right otolith.

proper cut resulted in annuli that were clearly defined and delineated. Once cut, the otolith section was placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400°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 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 — By convention, a birthdate of January 1 is assigned to all Northern Hemisphere fish species. The Age and Growth Lab uses a system of age determination that assigns age class according to the date of sacrifice with respect to this international accepted birthdate and the timing of annulus formation, which occurs in Virginia's waters between the months of February and April. Using this method, a fish sacrificed in January before annulus formation with three visible annuli will be assigned the same age as a fish with four visible annuli sacrificed in July after annulus formation. Once the reader has decided how many annuli are visible on the ageing structure, the year class is assigned. The year class designation, or age, is written first followed by the actual number of annuli visible listed within brackets (e.g. 3(3)). The presence of a "+" after the number in the brackets indicates new growth, or "plus growth" visible on the structure's margin.

Two different readers aged all samples 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 2) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses.

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.

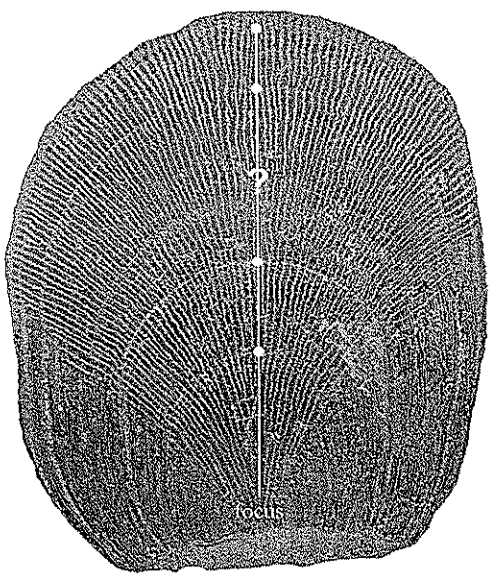


Figure 2. Scale impression of a 590 mm female summer flounder collected in November and aged as 4-years-old with scales. The question mark is located at a possible "3rd" annulus.

Otoliths – Sectioned otoliths were aged by two different readers using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 3).

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.

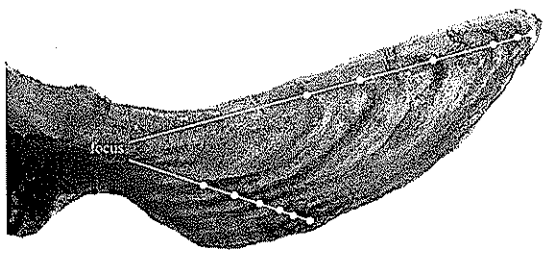


Figure 3. Otolith section from a 590 mm, 6-year-old female summer flounder collected in November. Same fish as Figure 2.

Comparison Tests — A random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the scales and otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and

time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Scales — Measurements of reader self-precision was low for Reader 1 (CV = 8.0% and fair for Reader 2 (CV = 4.3%). There was evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 52.1$, df = 22, $P = 0.0003$). In Figure 4 we present a graph of the results for between-reader scale ageing precision. The average between-reader coefficient of variation (CV) of 8.2% was relatively high. The between-reader agreement for scale for one year or less was 94.0% of all aged fish. Such a high agreement between the readers and the high CV for Reader 1 were partially due to the sample being dominated by younger fish.

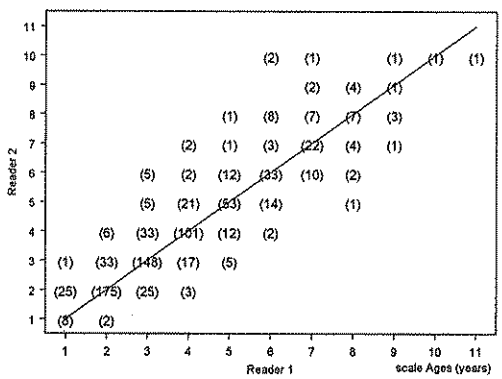


Figure 4. Between-reader comparison of scale age estimates for summer flounder in 2006.

Of the 826 fish aged with scales, 11 age-classes (1 and 11) were represented (Table 1). The average scale age was 3.8 years, and the standard deviation and standard error were 1.83 and 0.06, respectively.

Year-class data (Figure 5) indicate that recruitment into the fishery began at age 1, which corresponds to the 2005 year-class for summer flounder caught in 2006. Year-class abundance was high for the 2002–2004 year-classes, but declined sharply in the 2001 year-class and remained low for the earlier years.

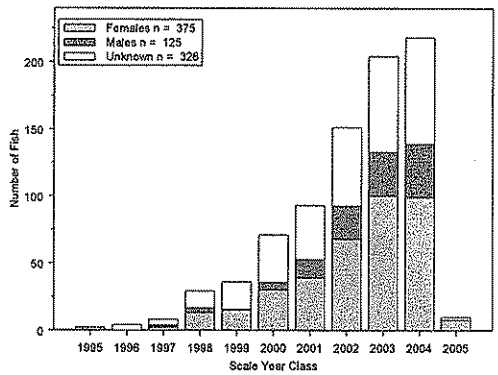


Figure 5. Scale year-class distribution for summer flounder collected in 2006. Distribution is broken down by sex.

There was no evidence of drift in scale age determination from Year 2000 precision fish. Agreement for one year or less was 96% for Reader 1 (CV = 6.4%, test of symmetry: $\chi^2 = 10$, df = 7, P = 0.1886) and 94% for Reader 2 (CV = 10.4%, test of symmetry: $\chi^2 = 8.6$, df = 8, P = 0.3772).

Otoliths — Measurements of reader self-precision were good for Reader 1 (CV = 2.3%) and fair for Reader 2 (CV = 5.4%). There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 23.6$, df = 14, P = 0.0519). In Figure 6 we present a graph of the results for between-reader otolith ageing precision. The average between-reader coefficient of variation (CV) of 3.0% was not significant.

Of the 541 fish aged with otoliths, 11 age-classes (2 to 12) were represented. The average age for the sample was 3.8 years. The standard deviation and standard error were 1.83 and 0.06, respectively.

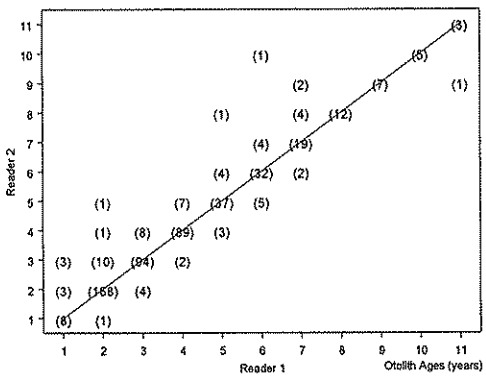


Figure 6. Between-reader comparison of otolith age estimates for summer flounder in 2006.

There was no evidence of drift in otolith age determination from Year 2000 precision fish. Agreement was 98% for Reader 1 (CV = 0.3%, test of symmetry: $\chi^2 = 1$, df = 1, P = 0.3173) and 84% for Reader 2 (CV = 4.5%, test of symmetry: $\chi^2 = 6$, df = 4, P = 0.1992).

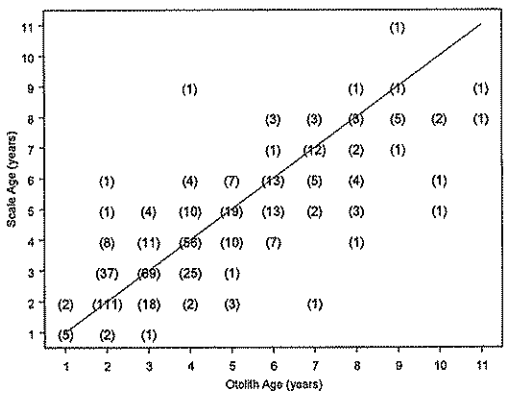


Figure 7. Comparison of otolith and scale age estimates for summer flounder in 2006.

Comparison of Scale and Otolith Ages — Otolith and scales ages were similar, with an average CV of 10.5% for the 496 fish for which both otoliths and scales were aged. There was evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 42.7$, df = 26, P = 0.0208). In Figure 7 we present a graph of the results for between-reader otolith/scale ageing precision. There was some evidence of bias between otolith and scale ages for the oldest fish in the sample (Figure 8), but this could be due to the extremely small number of fish in these age categories.

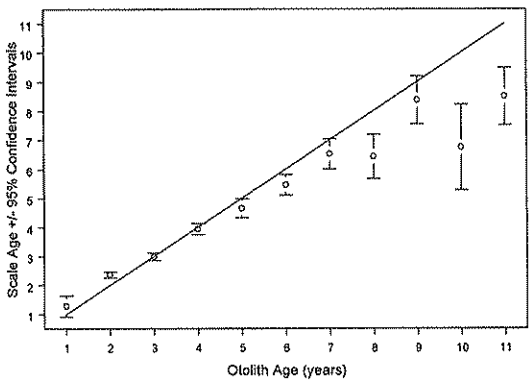


Figure 8. Age-bias plot for summer flounder scale and otolith age estimates in 2006.

Age-Length-Key — In Table 2 we present an age-length-key that 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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Hoenig, J.M., M.J. Morgan, and C.A. Brown. 1995. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

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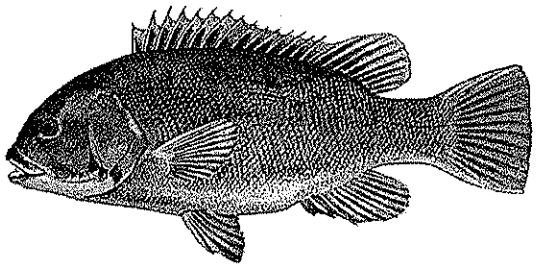
Table 1. The number of summer flounder assigned to each total length-at-age category for 826 fish sampled for scale-age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)											
	1	2	3	4	5	6	7	8	9	10	11	Total
12 - 12.99	0	0	1	0	0	0	0	0	0	0	0	1
13 - 13.99	1	19	13	2	0	0	0	0	0	0	0	35
14 - 14.99	2	53	30	9	1	0	0	0	0	0	0	95
15 - 15.99	2	63	39	20	9	1	0	0	0	0	0	134
16 - 16.99	1	47	44	14	7	4	2	1	0	0	0	120
17 - 17.99	4	23	28	28	8	2	0	1	1	0	0	95
18 - 18.99	0	6	19	24	14	6	1	1	0	0	0	71
19 - 19.99	0	2	12	20	5	5	0	1	1	0	0	46
20 - 20.99	0	3	9	14	13	6	2	2	0	0	0	49
21 - 21.99	0	2	5	11	8	11	3	3	0	0	0	43
22 - 22.99	0	0	3	4	13	10	3	2	0	1	0	36
23 - 23.99	0	0	1	2	11	13	5	4	2	0	0	38
24 - 24.99	0	0	0	1	3	9	6	2	1	0	0	22
25 - 25.99	0	0	0	2	1	2	7	5	0	0	1	18
26 - 26.99	0	0	0	0	0	2	6	5	2	1	0	16
27 - 27.99	0	0	0	0	0	0	0	1	1	2	0	4
28 - 28.99	0	0	0	0	0	0	1	0	0	0	0	1
29 - 29.99	0	0	0	0	0	0	0	1	0	0	1	2
Total	10	218	204	151	93	71	36	29	8	4	2	826

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-interval, based on scale ages for summer flounder sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)											
	1	2	3	4	5	6	7	8	9	10	11	N
12 - 12.99	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1
13 - 13.99	0.029	0.543	0.371	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	35
14 - 14.99	0.021	0.558	0.316	0.095	0.011	0.000	0.000	0.000	0.000	0.000	0.000	95
15 - 15.99	0.015	0.470	0.291	0.149	0.067	0.007	0.000	0.000	0.000	0.000	0.000	134
16 - 16.99	0.008	0.392	0.367	0.117	0.058	0.033	0.017	0.008	0.000	0.000	0.000	120
17 - 17.99	0.042	0.242	0.295	0.295	0.084	0.021	0.000	0.011	0.011	0.000	0.000	95
18 - 18.99	0.000	0.085	0.268	0.338	0.197	0.085	0.014	0.014	0.000	0.000	0.000	71
19 - 19.99	0.000	0.043	0.261	0.435	0.109	0.109	0.000	0.022	0.022	0.000	0.000	46
20 - 20.99	0.000	0.061	0.184	0.286	0.265	0.122	0.041	0.041	0.000	0.000	0.000	49
21 - 21.99	0.000	0.047	0.116	0.256	0.186	0.256	0.070	0.070	0.000	0.000	0.000	43
22 - 22.99	0.000	0.000	0.083	0.111	0.361	0.278	0.083	0.056	0.000	0.028	0.000	36
23 - 23.99	0.000	0.000	0.026	0.053	0.289	0.342	0.132	0.105	0.053	0.000	0.000	38
24 - 24.99	0.000	0.000	0.000	0.045	0.136	0.409	0.273	0.091	0.045	0.000	0.000	22
25 - 25.99	0.000	0.000	0.000	0.111	0.056	0.111	0.389	0.278	0.000	0.000	0.056	18
26 - 26.99	0.000	0.000	0.000	0.000	0.000	0.125	0.375	0.313	0.125	0.063	0.000	16
27 - 27.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.250	0.500	0.000	4
28 - 28.99	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1
29 - 29.99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.500	2
										Sample Size		826

Chapter 12
Tautog



Tautoga onitis

INTRODUCTION

A total of 503 tautog, *Tautoga onitis*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. Of 503 fish aged, 492 fish had both opercula and otoliths, 6 fish had opercula only, and 5 fish had otoliths only. Our results and analyses are based on operculum ages, unless otherwise noted, to allow our data to be directly comparable to other tautog age and growth studies. The average operculum age for the sample was 4.5 years, and the standard deviation and standard error were 2.28 and 0.10, respectively. Thirteen age-classes (2-14) were represented, comprising fish from the 1992 through 2004 year-classes.

METHODS

Handling of collection — 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 sample number. All otoliths were stored dry in labeled cell well plates, while opercula were stored frozen in their original coin envelopes until processed.

Preparation —

Opercula – Tautog opercula were boiled for several minutes to remove any attached skin and muscle tissue. After boiling, opercula were examined to determine whether they were collected whole or in some way damaged. Opercula were allowed to dry and finally stored in new labeled coin envelopes.

Otoliths – Because of the small size of a tautog otolith, it required extra steps in preparation for ageing. An otolith was first baked in a Thermolyne 1400 furnace at 400°C for one to two minutes until it turned a medium brown color (caramel). The location of the core of the otolith was marked with a felt pen and the entire otolith was embedded in Loctite 349 adhesive, placed under UV light, and allowed to harden overnight. The otolith was then transversely sectioned through the felt pen mark with a low speed Buehler Isomet saw equipped with double wafering blades separated by a 0.5 mm spacer. The sectioned side of the otolith was then placed face down in a drop of Loctite 349 photo-active adhesive on a labeled glass slide and placed under ultraviolet light to allow the adhesive to harden (approximately ten minutes). The otolith section was then polished using a Buehler Ecomet 3 variable speed grinder-polisher with Mark V Laboratory 30-micron polishing film. After polishing, a thin layer of Flo-texx mounting medium was applied to the otolith section to increase light transmission through the translucent zones, which provided enhanced contrast and greater readability.

Readings — Opercula were aged on a light table with no magnification (Figure 1). Sectioned otoliths were aged by two different readers using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 2).

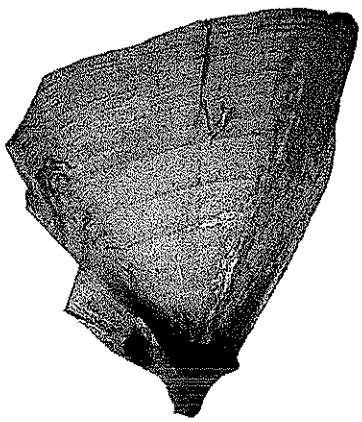


Figure 1. Operculum from a 13 year-old male tautog.

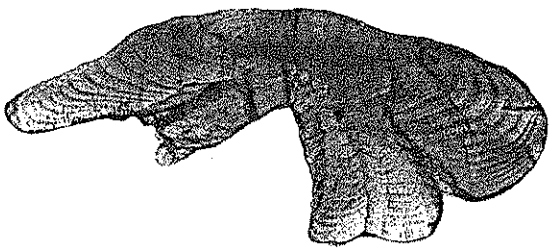


Figure 2. Otolith section from a 13 year-old male tautog. Same fish as Figure 1.

Two different readers aged all samples 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 random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the scales and otoliths of 60 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

Opercula — Measurements of reader self-precision were fair, with both readers able to reproduce the ages of previously read opercula (Reader 1's CV = 5.4% and Reader 2's CV = 4.8%). In Figure 3 we present a graph of the results for between-reader operculum ageing precision. There was evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 46.2$, df = 25, $P = 0.0060$). The average between-reader coefficient of variation (CV) of 7.3% and was relatively high but lower than the CV of 9.2% in 2005. The between-reader agreement for operculum for one year or less was 95.0% of all aged fish. The high agreement between the readers and the high CVs were

partially due to the sample dominated by younger fish.

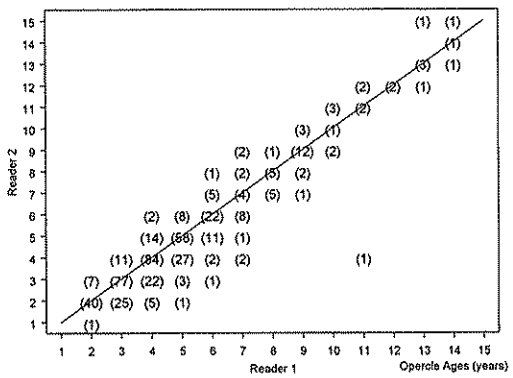


Figure 3. Between-reader comparison of operculum age estimates for tautog in 2006.

The average operculum age for the sample was 4.5 years, and the standard deviation and standard error were 2.28 and 0.10, respectively. Year-class data (Figure 4) indicate that recruitment into the fishery occurred at age 2, which corresponds to the 2004 year-class for tautog caught in 2006. Year-class abundance was high for the 2001–2004 year-classes.

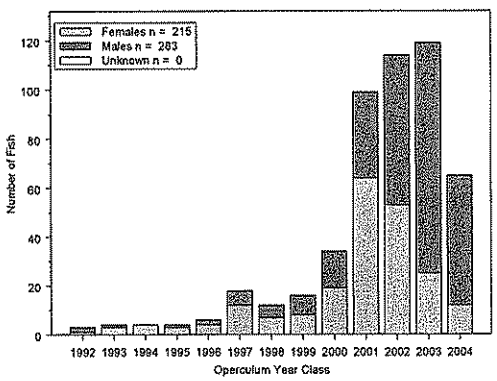


Figure 4. Operculum year-class distribution for tautog collected in 2006. Distributions are broken down by sex.

There was no evidence of drift in operculum age determination from Year 2000 precision fish. Agreement for one year or less was 94% for Reader 1 (CV = 7.0%, test of symmetry: $\chi^2 = 14.8$, df = 12, P = 0.2526) and 98% for Reader 2 (CV = 4.4%, test of symmetry: $\chi^2 = 11.7$, df = 9, P = 0.2328).

Otoliths — Measurements of reader self-precision were good, with both readers able to reproduce the ages of previously read otoliths (Reader 1's CV = 2.0% and Reader 2's CV = 0.9%). There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 23.3$, df = 17, P = 0.1387). In Figure 5 we present a graph of the results for between-reader otolith ageing precision. The average between-reader coefficient of variation (CV) of 1.7% was not significant and lower than the CV of 4.1% in 2005.

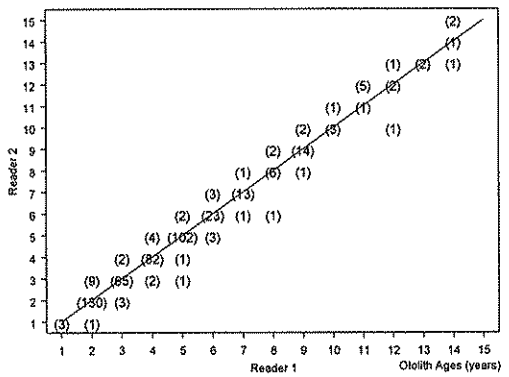


Figure 5. Between-reader comparison of otolith age estimates for tautog in 2006.

Of the 497 fish aged with otoliths, 15 age-classes (1 through 15) were represented. The average age for the sample was 4.3 years. The standard deviation and standard error were 2.42 and 0.11, respectively.

There was no evidence of drift in otolith age determination from Year 2000 precision

fish. Agreement was 92% for Reader 1 (CV = 0.9%, test of symmetry: $\chi^2 = 4$, df = 2, P = 0.1353) and 84% for Reader 2 (CV = 1.8%, test of symmetry: $\chi^2 = 8$, df = 5, P = 0.1562).

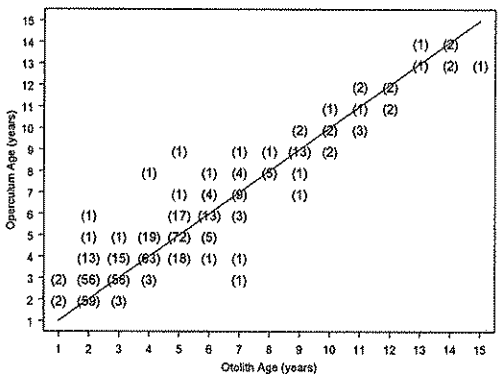


Figure 6. Comparison of otolith and operculum age estimates for tautog in 2006.

Comparison of Operculum and Otolith Ages — The between-structure average CV was 9.0%. There was evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 95.7$, df = 26, $P < 0.0001$). Operculum were assigned a lower age than otoliths for 9.6% of the fish and 30% of the time were operculum assigned a higher age than otoliths (Figure 6). There was also evidence of bias between otolith and operculum ages using an age bias plot (Figure 7), again with operculum generally assigned higher ages for younger fish and lower ages for older fish than otoliths age estimates.

Age-Length-Key — In Table 2 we present an age-length-key that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

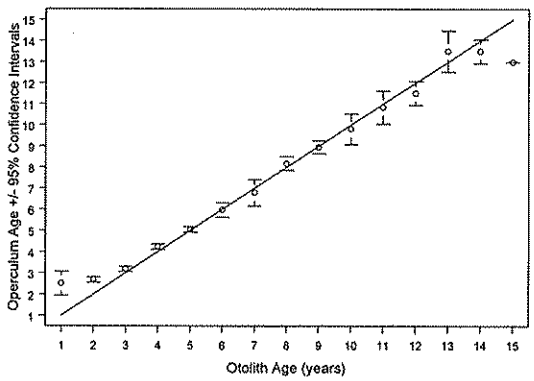


Figure 7. Age-bias plot for tautog otolith and operculum age estimates in 2006.

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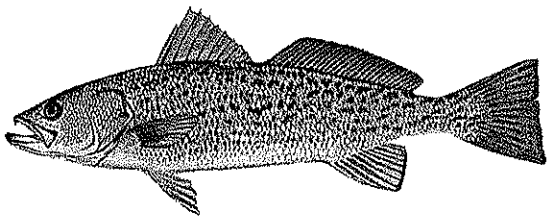
Table 1. The number of tautog assigned to each total length-at-age category for 498 fish sampled for operculum-age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)													
	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
11 - 11.99	0	3	0	0	0	0	0	0	0	0	0	0	0	3
12 - 12.99	0	2	2	2	0	0	0	0	0	0	0	0	0	6
13 - 13.99	25	24	15	7	1	0	0	0	0	0	0	0	0	72
14 - 14.99	28	46	31	16	0	0	0	0	0	0	0	0	0	121
15 - 15.99	12	28	29	18	3	0	0	1	0	0	0	0	0	91
16 - 16.99	0	11	17	22	9	4	0	0	0	0	0	0	0	63
17 - 17.99	0	3	9	19	14	3	7	2	1	0	1	1	0	60
18 - 18.99	0	2	7	11	5	5	3	5	3	2	1	0	0	44
19 - 19.99	0	0	4	2	1	4	0	4	3	2	1	2	2	25
20 - 20.99	0	0	0	0	0	0	2	4	0	0	1	0	0	7
21 - 21.99	0	0	0	1	0	0	0	1	0	0	0	1	1	4
22 - 22.99	0	0	0	0	1	0	0	1	0	0	0	0	0	2
Total	65	119	114	98	34	16	12	18	7	4	4	4	3	498

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-class, based on operculum-ages for tautog sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)													
	2	3	4	5	6	7	8	9	10	11	12	13	14	N
11 - 11.99	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3
12 - 12.99	0.000	0.333	0.333	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6
13 - 13.99	0.347	0.333	0.208	0.097	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	72
14 - 14.99	0.231	0.380	0.256	0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	121
15 - 15.99	0.132	0.308	0.319	0.198	0.033	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	91
16 - 16.99	0.000	0.175	0.270	0.349	0.143	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	63
17 - 17.99	0.000	0.050	0.150	0.317	0.233	0.050	0.117	0.033	0.017	0.000	0.017	0.017	0.000	60
18 - 18.99	0.000	0.045	0.159	0.250	0.114	0.114	0.068	0.114	0.068	0.045	0.023	0.000	0.000	44
19 - 19.99	0.000	0.000	0.160	0.080	0.040	0.160	0.000	0.160	0.120	0.080	0.040	0.080	0.080	25
20 - 20.99	0.000	0.000	0.000	0.000	0.000	0.000	0.286	0.571	0.000	0.000	0.143	0.000	0.000	7
21 - 21.99	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.250	0.250	4
22 - 22.99	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	2
												Sample size		498

Chapter 13



Weakfish
Cynoscion regalis

INTRODUCTION

A total of 614 weakfish, *Cynoscion regalis*, was collected by the VMRC's Biological Sampling Program for age and growth analysis in 2006. The average age was 2.9 years old, and the standard deviation and standard error were 0.84 and 0.04, respectively. Five age classes (1 to 5) were represented, comprising fish from the 2001 through 2005 year-classes, with fish primarily from the 2003 year-classes.

METHODS

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 in labeled cell well trays.

Preparation — The first step in otolith preparation was to grind down the otolith in a transverse plane to its core using a Hillquist thin section machine's 320-mesh diamond cup wheel. To prevent distortion of the reading surface, the otolith was ground exactly perpendicular to the reading plane. The otolith's ground surface was then placed face down in a drop of Loctite 349 photo-active adhesive on a labeled glass slide and placed under ultraviolet light to allow the adhesive to harden. The Hillquist thin section machine's cup wheel was used again to grind the otolith, embedded in Loctite, to a thickness of 0.3 to 0.5 mm. Finally, a thin layer of Flo-texx mounting medium was applied to the otolith section to increase light transmission through the translucent zones, which provided enhanced contrast and greater readability.

Readings — Two different readers aged all sectioned otoliths using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification (Figure 1). Each reader aged all of the otolith sections using ageing criteria listed in Lowerre-Barbieri et al. (1994). 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.

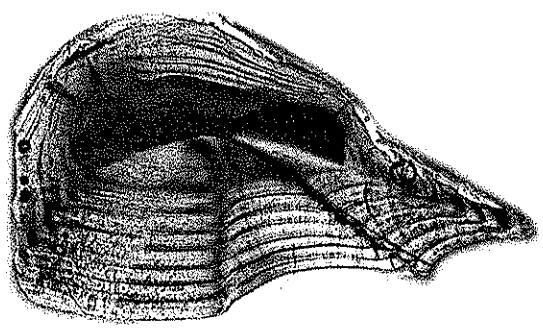


Figure 1. Sectioned otolith from a 7 year old female weakfish.

When the readers were unable to agree on a final age, the fish was excluded from further analysis.

Comparison Tests — A random sub-sample of 50 fish was selected for second readings to measure reader precision and age reproducibility using the coefficient of variance (CV). Age estimates from Reader 1 were plotted against age estimates from Reader 2 to assess deviation from 1:1 equivalence (Campana et al. 1995). Also, to detect any changes or drift in our ageing methods, both readers re-aged the otoliths of 50 randomly selected fish previously aged in 2000. A test for symmetry was used to detect any systematic difference between the two readers and time-series bias between the current readings and previous readings of Year 2000 precision fish (Hoenig et al. 1995). We considered a reader to be biased if the readings revealed consistent over or under ageing.

RESULTS

The measurement of reader self-precision was high for both readers (Both readers had 0% CVs). There was no evidence of systematic disagreement between reader 1 and reader 2 (test of symmetry: $\chi^2 = 2$, df = 2, P = 0.3679). Figure 2 illustrates the between readers' precision of age estimates. The average coefficient of variation (CV) of 0.2% was not significant. There was no evidence of drift in age determination from Year 2000 precision fish. Agreement was 94% for Reader 1 (CV = 1.2%, test of symmetry: $\chi^2 = 3$, df = 3, P = 0.3916) and 84% for Reader 2 (CV = 3.5%, test of symmetry: $\chi^2 = 6$, df = 6, P = 0.4232).

Of the 614 fish aged with otoliths, 5 age classes were represented (Table 1). The average age was 2.9 years old, and the standard deviation and standard error were 0.84 and 0.04, respectively.

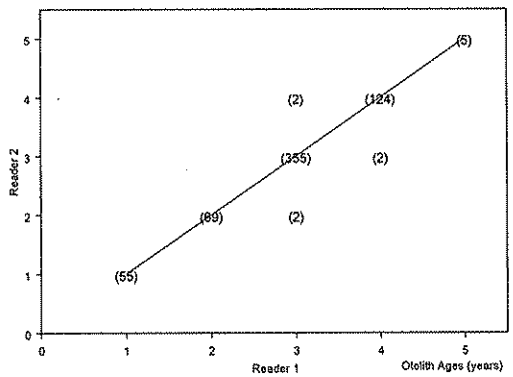


Figure 2. Between-reader comparison of otolith age estimates for weakfish in 2006.

Year-class data (Figure 3) show that the fishery was comprised of 5 year-classes, comprising fish from the 2001-2005 year-classes, with fish primarily from the 2003 year-classes.

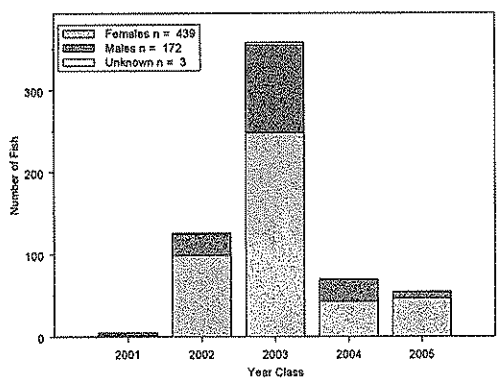


Figure 3. Year-class frequency distribution for weakfish collected for ageing in 2006. Distribution is broken down by sex.

Age-Length-Key — In Table 2 we present an age-length-key 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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Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124:131-138.

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Lowerre-Barbieri, S.K., M.E. Chittenden Jr., and C.M. Jones. 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. Fish Bull. 92:555-568.

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Table 1. The number of weakfish assigned to each total length-at-age category for 614 fish sampled for age determination in Virginia during 2006 (no length for 1 fis

Length 1-inch intervals	Age (years)					
	1	2	3	4	5	Total
8 - 8.99	1	2	0	0	0	3
9 - 9.99	0	25	8	0	0	33
10 - 10.99	1	13	37	1	0	52
11 - 11.99	1	7	49	3	0	60
12 - 12.99	11	1	19	5	0	36
13 - 13.99	10	2	18	1	0	31
14 - 14.99	12	3	19	8	1	43
15 - 15.99	14	4	18	26	0	62
16 - 16.99	5	5	47	22	0	79
17 - 17.99	0	3	42	15	4	64
18 - 18.99	0	2	34	14	0	50
19 - 19.99	0	2	30	15	0	47
20 - 20.99	0	0	16	7	0	23
21 - 21.99	0	1	9	5	0	15
22 - 22.99	0	0	7	1	0	8
23 - 23.99	0	0	3	2	0	5
24 - 24.99	0	0	1	0	0	1
29 - 29.99	0	0	0	1	0	1
Total	55	70	357	126	5	613

Table 2. Age-Length key, as proportions-at-age in each 1 inch length-interval, based on otolith ages for weakfish sampled for age determination in Virginia during 2006.

Length 1-inch intervals	Age (years)					
	1	2	3	4	5	N
8 - 8.99	0.333	0.667	0.000	0.000	0.000	3
9 - 9.99	0.000	0.758	0.242	0.000	0.000	33
10 - 10.99	0.019	0.250	0.712	0.019	0.000	52
11 - 11.99	0.017	0.117	0.817	0.050	0.000	60
12 - 12.99	0.306	0.028	0.528	0.139	0.000	36
13 - 13.99	0.323	0.065	0.581	0.032	0.000	31
14 - 14.99	0.279	0.070	0.442	0.186	0.023	43
15 - 15.99	0.226	0.065	0.290	0.419	0.000	62
16 - 16.99	0.063	0.063	0.595	0.278	0.000	79
17 - 17.99	0.000	0.047	0.656	0.234	0.063	64
18 - 18.99	0.000	0.040	0.680	0.280	0.000	50
19 - 19.99	0.000	0.043	0.638	0.319	0.000	47
20 - 20.99	0.000	0.000	0.696	0.304	0.000	23
21 - 21.99	0.000	0.067	0.600	0.333	0.000	15
22 - 22.99	0.000	0.000	0.875	0.125	0.000	8
23 - 23.99	0.000	0.000	0.600	0.400	0.000	5
24 - 24.99	0.000	0.000	1.000	0.000	0.000	1
29 - 29.99	0.000	0.000	0.000	1.000	0.000	1
				Sample size		613

Chapter 14

Sample size for ageing

INTRODUCTION

Age and Growth Laboratory of Center for Quantitative Fisheries Ecology (CQFE) at Old Dominion University has been funded by Virginia Marine Resources Commission (VMRC) since 1998. The lab ages 13 marine finfish species and reports age information of these species to VMRC annually. The age information is used to construct age-length keys (ALK) for estimating age composition in the catch of each species by VMRC.

Age composition of the catch is one of critical inputs to fish stock assessment, therefore, we have made substantial effort to increase the quality of the estimation of age composition. Precision is used to measure the quality of the estimation of age composition and relies on the number of fish aged. Theoretically, the more fish that are aged, the higher the precision that can be achieved. However, ageing more fish is also more expensive. Therefore, the level of precision and the cost to age fish have to be balanced in practice, and an effective sample size for ageing should be ascertained a priori to reach an acceptable level of precision. For example, Aanes and Pennington (2003) reported that the sample size for ageing northeast Arctic cod could be reduced significantly without a significant loss in precision.

In this study, we were to calculate the effective sample size for ageing each of 10 species with acceptable precision. Our objectives were to: 1) calculate numbers of fish need to be aged using a series of coefficients of variance (*CV*) for each age of a species; 2) to determine the number of fish necessary to age to reach acceptable *CV*s for all ages of a species; 3) discuss significance of determination of effective samples for ageing in fisheries management.

METHODS

The species in this study were Atlantic croaker *Micropogonias undulatus*, bluefish *Pomatomus saltatrix*, spadefish *Chaetodipterus faber*, Spanish mackerel *Scomberomorous maculatus*, spot *Leiostomus xanthurus*, spotted seatrout *Cynoscion nebulosus*, striped bass *Morone saxatilis*, summer flounder *Paralichthys dentatus*, tautog *Tautoga onitis*, and weakfish *Cynoscion regalis*. We didn't include black drum *Pogonias cromis*, cobia *Rachycentron canadum* and red drum *Sciaenops ocellatus* because few specimens of these species were collected from 1999 to 2005. The methods for handling and processing fish, preparing and reading hard parts should be referred to in the previous chapters in this report.

First, we used a proportional allocation measured by the coefficient of variance (*CV*) method (Quinn and Deriso 1999) to calculate sample sizes with specific *CV*s for all ages of each species. Then, we decided an effective sample size which would give acceptable *CV*s for all ages of the species. The methods for all 10 species are the same as above. Therefore, we will not repeat this description for each species.

1. Calculation of *sample sizes for ageing*

We made a matrix with fish age in columns and total length in rows by combining all the fish we aged in each 1-inch length interval collected during the past several years. The number of years for making the matrix is species specific. This information served as a pilot study from which all the parameters were calculated. An allocation method was used to calculate sample sizes with specific *CV*s (Quinn and Deriso 1999). The specific calculations are as follows:

$$\alpha_l = A_l / A, \qquad (1)$$

where α_l is the proportion of fish of length l, A is the total number of fish we aged from 1999 to 2005 and A_l is the sum of the fish of length l in A.

$$\theta_{la} = A_{la} / A_l, \qquad (2)$$

where θ_{la} is the proportion of fish of length l and age a and A_{la} is the number of fish of length l and age a in A.

$$\theta_a = \sum_l \alpha_l \theta_{la}, \qquad (3)$$

where θ_a is the proportion of age a fish.

$$V_a = \sum_l \alpha_l \theta_{la} (1 - \theta_{la}) \qquad (4)$$

$$B_a = \sum_l \alpha_l \theta_{la} (\theta_{la} - \theta_a)^2, \qquad (5)$$

where V_a and B_a are components of variance within length intervals for proportional allocation and between length intervals, respectively.

Therefore, the coefficient of variance can be calculated as:

$$CV^2 = \left(\frac{V_a}{A} + \frac{B_a}{L} \right) / \theta_a^2, \qquad (6)$$

where L is the sample size for estimating length intervals of the catch of a species sampled by VMRC each year.

Solving for A in Equation 6, we have

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}. \qquad (7)$$

In this study, Equation 7 was used to calculate sample size for ageing that gives the particular age composition estimate (θ_a) with a specific level of precision (*CV*). Finally, the number of fish of length l needed for ageing was obtained from Equation 1 as follows (proportional allocation):

$$A_l = A \alpha_l \qquad (8)$$

There are two assumptions under this method:
1) The length intervals derived from L represent the length intervals of the catch;
2) A_l is taken randomly from L_l (number of fish of length l in L). This sampling scheme is called as two-stage random sampling (Quinn and Deriso 1999).

2. *Decision of a sample size for ageing using specific CVs*

As described above, the sample size A is dependent on not only V_a and B_a but *CV* as well. Therefore, the *CV*s were chosen in advance. In this study, we assumed that *CV*s below 0.25 were acceptable. Therefore, we calculated a series of *A*s for each age by applying *CV*s ranging from 0.05 up to 0.25 with each increment of 0.01 to Equation 7.

Then, we decided an effective sample size *A* by comparing the series of the *A*s derived using the different *CV*s within each age and among all ages of a species. An effective sample size was defined as a minimum sample size with an acceptable *CV* beyond which increasing number of fish to age will not decrease the *CV* significantly.

Length frequency intervals were developed by every 1-inch using the total number of fish sent us by VMRC in 2006. Number of fish aged in each length interval within a species was estimated using the proportional allocation (Equation 8). When the total number of fish received from VMRC was significantly larger than estimated for ageing, we randomly sampled the fish by length interval to obtain estimated sample size for that particular length interval. When a fish randomly selected couldn't be aged due to a missing or damaged hard part, then another fish was randomly selected to replace it.

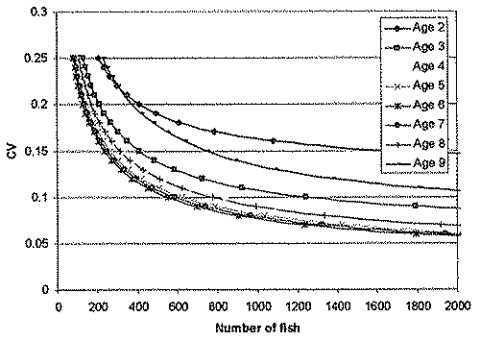
RESULTS

1. *Sample size for ageing a species*

We reported the series of *A*s derived using different *CV*s as a matrix for each of 10 species (Appendix). The matrix was used to determine the sample size *A* for the species. The detailed descriptions on how to decide *A* for a particular species are followed below.

1) Atlantic croaker

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2001). The *CV*s for Age 3-8 didn't decrease significantly and for Age 2 and 9 were within an acceptable range (<0.25) after the number of fish for ageing reached about 300 (Figure 1). Therefore, we decided that the effective sample size was 336 for Atlantic croaker collected in 2006. This sample size achieved the *CV*s of 0.22, 0.17, 0.15, 0.14, 0.13, 0.13, 0.15, and 0.21 for Age 2, 3, 4, 5,

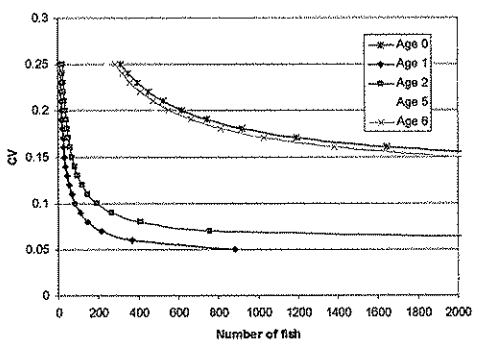


6, 7, 8, and 9, respectively (Appendix: Table 1). Age 2-9 fish took about 92% of the total catch of croaker from 1999 to 2005.

Figure 1. Relationship between number of fish for ageing and *CV* for Age 2-9 of Atlantic croaker.

2) Bluefish

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2001). The *CV*s for Age 1 and 2 didn't decrease significantly and for Age 0, 5 and 6 were within an acceptable range (<0.25) after the number of fish for ageing reached about 300 (Figure 2). Therefore, we decided that the effective sample size was 321 for bluefish collected in 2006. This sample size achieved the *CV*s of 0.25, 0.07, 0.09, 0.23, and 0.24 for Age 0, 1, 2, 5, and 6, respectively



(Appendix: Table 2). These age groups of fish took about 89% of the total catch of bluefish from 1999 to 2005.

Figure 2. Relationship between number of fish for ageing and *CV* for Age 0-2 and 5-6 of bluefish.

3) Spadefish

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2001). The *CV*s for Age 1-3 didn't decrease significantly and for Age 4-8 were within an acceptable range (<0.25) after the number of fish for ageing reached about 300 (Figure 3). Therefore, we decided that the effective sample size was 328 for spadefish collected in 2006. This sample size achieved the *CV*s of 0.11, 0.09, 0.13, 0.24, 0.24, 0.25, and 0.25 for Age 1-8, respectively (Appendix: Table 3). These age groups of fish took about 85% of the total catch of spadefish from 1999 to 2005.

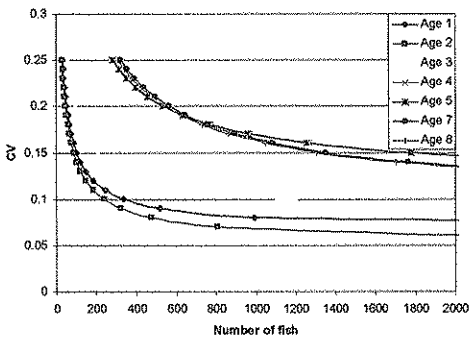


Figure 3. Relationship between number of fish for ageing and *CV* for Age 1-8 of spadefish.

4) Spanish mackerel

The matrix of age frequency by length intervals was made from the data collected from 2003 to 2005. The *CV*s for Age 1 and 2 didn't decrease significantly and for Age 3 and 4 were within an acceptable range (<0.25) after the number of fish for ageing reached about 300 (Figure 4). Therefore, we decided that the effective sample size was 291 for Spanish mackerel collected in 2006. This sample size achieved the *CV*s of 0.04, 0.13, 0.22, and 0.25 for Age 1-4, respectively (Appendix: Table 4). These age groups of fish took about 95% of the total catch of Spanish mackerel from 1999 to 2005.

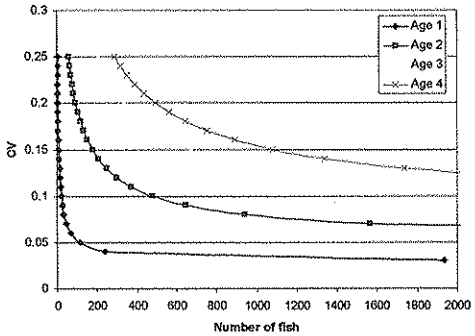


Figure 4. Relationship between number of fish for ageing and *CV* for Age 1-4 of Spanish mackerel.

5) Spot

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2001). The *CV*s for Age 1-3 didn't decrease significantly after the number of fish for ageing reached about 200 (Figure 5). We decided that the effective sample size 262 for spot collected in 2006. This sample size achieved the *CV*s of 0.05, 0.10, and 0.17 for Age 1-3, respectively (Appendix: Table 5). These age groups of fish took about 95% of the total catch of spot from 1999 to 2005.

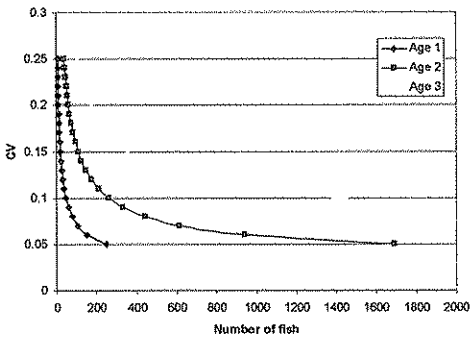


Figure 5. Relationship between number of fish for ageing and *CV* for Age 1-3 of spot.

6) *Spotted seatrout*

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2000 and 2001). The *CV*s for Age 0-2 didn't decrease significantly and for Age 3 was within an acceptable range (<0.25) after the number of fish for ageing reached about 200 (Figure 6). Therefore, we decided that the effective sample size was 255 for spotted seatrout collected in 2006. This sample size achieved the *CV*s of 0.14, 0.06, 0.11, and 0.23 for Age 0-3, respectively (Appendix: Table 6). These age groups of fish took about 94% of the total catch of spotted seatrout from 1999 to 2005.

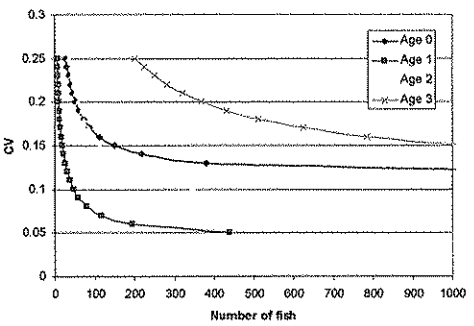


Figure 6. Relationship between number of fish for ageing and *CV* for Age 0-3 of spotted seatrout.

7) Striped bass

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005. The *CV*s for Age 4-12 didn't decrease significantly and for Age 3 and 13-14 were within an acceptable range (<0.25) after the number of fish for ageing reached about 900 (Figure 7). Therefore, we decided that the effective sample size was 905 for striped bass collected in 2006. This sample size achieved the *CV*s of 0.19, 0.13, 0.11, 0.10, 0.09, 0.09, 0.08, 0.09, 0.11, 0.14, 0.19 and 0.24 for Age 3-14, respectively (Appendix: Table 7). These age groups of fish took about 97% of the total catch of striped bass from 1999 to 2005.

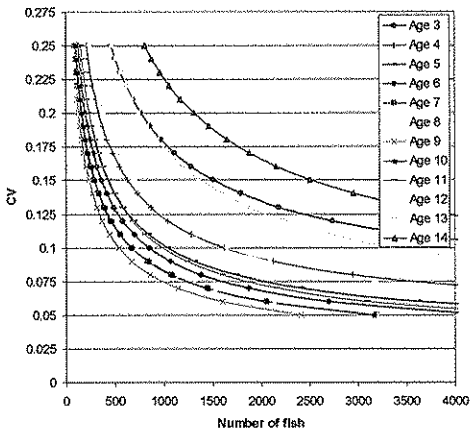


Figure 7. Relationship between number of fish for ageing and *CV* for Age 3-14 of striped bass.

8) Summer flounder

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005. The *CV*s for Age 1-6 didn't decrease significantly and for Age 7 was within an acceptable range (<0.25) after the number of fish for ageing reached about 700 (Figure 8). Therefore, we decided that the effective sample size was 722 for summer flounder collected in 2006. This sample size achieved the *CV*s of 0.08, 0.06, 0.07, 0.09, 0.12, 0.18, and 0.25 for Age 1-7, respectively (Appendix: Table 8). These age

groups of fish took about 97% of the total catch of summer flounder from 1999 to 2005.

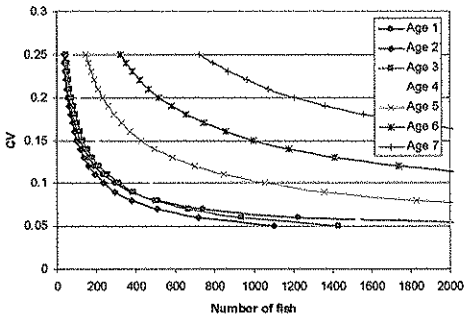


Figure 8. Relationship between number of fish for ageing and *CV* for Age 1-7 of summer flounder.

9) Tautog

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005. The *CV*s for Age 2-6 didn't decrease significantly and for Age 7 were within an acceptable range (<0.25) after the number of fish for ageing reached about 500 (Figure 9). Therefore, we decided that the effective sample size was 494 for tautog collected in 2006. This sample size achieved the *CV*s of 0.15, 0.09, 0.09, 0.11, 0.13, 0.20, and 0.22 for Age 2-8, respectively (Appendix: Table 9). These age groups of fish took about 88% of the total catch of tautog from 1999 to 2005.

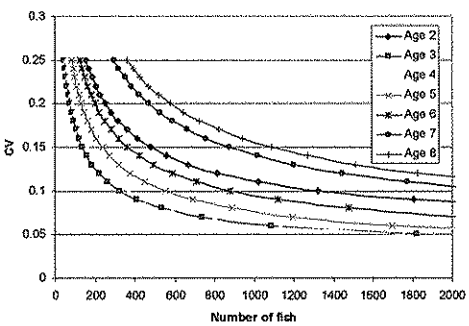


Figure 9. Relationship between number of fish for ageing and *CV* for Age 2-8 of tautog.

10) Weakfish

The matrix of age frequency by length intervals was made from the data collected from 1999 to 2005 (but not from 2001). The *CV*s for Age 1-5 didn't decrease significantly and for Age 6 was within an acceptable range (<0.25) after the number of fish for ageing reached about 600 (Figure 10). Therefore, we decided that the effective sample size was 594 for weakfish collected in 2006. This sample size achieved the *CV*s of 0.11, 0.06, 0.08, 0.10, 0.14, and 0.24 for Age 1-6, respectively (Appendix: Table 10). These age groups of fish took about 97% of the total catch of weakfish from 1999 to 2005.

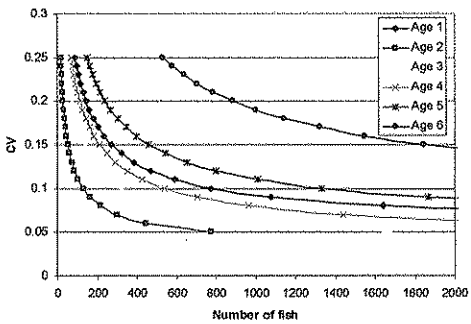


Figure 10. Relationship between number of fish for ageing and *CV* for Age 1-6 of weakfish.

2. *Sample size for each length interval within a species*

We received more Spanish mackerel, spot, spotted seatrout, striped bass, and summer flounder than were needed for ageing. We received 418 Spanish mackerel, 384 spot, 357 spotted seatrout, 1641 striped bass, and 1154 summer flounder in 2006. We aged 291 Spanish mackerel, 263 spot, and 256 spotted seatrout, 906 striped bass, and 826 summer flounder (Table 1). Scale-ages of striped bass and summer flounder were used for analysis in this study. Table 1 indicates

the sample size for each length interval and the number of fish randomly selected and aged in each length interval for those species in 2006.

DISCUSSION

The goal of estimating age-sample size is to age a minimum number of fish to obtain age compositions of the most important age groups in a catch with acceptable precision (Quinn et al. 1983). By knowing the specific sampling sizes for ageing with acceptable precision, we are able to not only monitor and improve the quality of estimates of age composition but also increase the efficiency of the ageing process. For example, we received a sample of 1154 summer flounder collected by VMRC in 2006. We estimated that 722 fish was an effective ageing sample size for summer flounder. We found that a larger sample size of 1154 would neither significantly increase the precision for the most important age groups nor include more important age groups with acceptable precision (Appendix: Table 8). Therefore, we reduced by more than one-third our ageing effort on this species (more than 400 fish less), while obtaining almost the same quality of estimates of age composition for summer flounder in 2006.

The ageing sample size rests on the number of age groups in a catch and variances within and between length intervals. Theoretically, when there are more age groups in a catch and larger variances within and between length intervals then we will require larger sample sizes. The multiple-year data used in our study would have more age groups and larger variances than any single year. In addition, we used number of fish we received from VMRC as L in Equation 7 to calculate the sample size for ageing. Theoretically, L is number of fish collected by VMRC to estimate the length frequency of the catch of a species, which is larger than the number of fish we received. Therefore, these factors make our estimate of ageing sample size more conservative. In other words, we aged more fish than we probably needed, guaranteeing an acceptable precision for an age in a particular species in 2006.

The ageing sample size estimated in this study is designed to develop one ALK with all spatial, temporal and other factors combined. When those factors influence age composition of a catch, ageing sample sizes should be estimated with incorporation of those factors. For example, VMRC developed 6 ALKs by area, season, and gear for commercial catch in 2006. Therefore, we can use these ALKs as a pilot study to estimate 6 effective sample sizes for ageing by area, season, and gear in 2007. This study is the first one to estimate sample size for ageing since the A&G Lab was established. We will continue to work on this issue in order to standardize ageing sample sizes for all the species we add in the future.

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

Table 1. Numbers of fish estimated for ageing and actually aged for Spanish mackerel, spot, spotted seatrout, striped bass, and summer flounder by every 1-inch length interval in 2006. Scale-ages were used for striped bass and summer flounder.

Length	Spanish mackerel		Spot		Spotted seatrout		Striped bass		Summer flounder	
interval	Estimated	Aged	Estimated	Aged	Estimated	Aged	Estimated	Aged	Estimated	Aged
6			10	10						
7			22	22						
8			57	57						
9			103	103	1	1				
10			37	38	5	5				
11	1	1	21	22	12	12				
12	0	0	9	9	30	30			1	1
13	3	3	3	2	25	25			22	35
14	19	18			17	17			81	95
15	60	60			13	13	1	1	116	134
16	63	64			13	13	0	0	105	120
17	49	50			27	27	0	0	88	95
18	23	23			13	13	8	9	65	71
19	13	13			24	24	14	10	49	46
20	9	9			18	18	8	11	39	49
21	20	19			14	14	14	15	38	43
22	15	15			9	9	21	21	28	36
23	6	6			12	12	33	33	30	38
24	5	5			11	11	45	44	23	22
25	1	1			5	5	42	43	16	18
26	1	1			2	2	51	51	16	16
27	2	2			4	4	39	39	4	4
28	0	0					38	38	1	1
29	0	0					23	22	1	2
30	1	1					17	18		
31							24	24		
32							36	36		
33							57	57		
34							74	74		
35							85	85		
36							114	114		
37							77	77		
38							26	26		
39							23	19		
40							11	15		
41							5	5		
42							10	10		
43							2	2		
44							3	2		
45							2	3		
46							0	0		
47							0	0		
48							0	0		
49							0	0		
50							1	1		
51							0	0		
52							0	0		
53							1	1		
Total	291	291	262	263	255	255	905	906	722	826

APPENDIX

Table 1. A series of sample sizes for ageing Atlantic croaker with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	1	2	3	4	5	6	7	8	9	10	11	12	13	15
0.05	-469	-670	-5751	10934	3542	2900	3209	6234	-5817	-7822	-7074	-4794	-15138	-17051
0.06	-472	-726	-33122	3960	2104	1790	1939	3065	-11267	-12327	-8488	-5205	-16418	-17218
0.07	-477	-805	7163	2258	1421	1232	1321	1915	105190	-38592	-11114	-5792	-18242	-17419
0.08	-481	-922	2980	1510	1034	907	966	1336	8137	26459	-17281	-6657	-20924	-17656
0.09	-487	-1103	1793	1097	790	698	741	995	3978	9091	-46577	-8016	-25107	-17934
0.10	-494	-1413	1241	841	626	555	587	774	2532	5244	52063	-10383	-32332	-18254
0.11	-501	-2049	926	668	508	452	478	622	1806	3573	15584	-15414	-47410	-18622
0.12	-509	-4043	724	545	422	376	397	512	1374	2649	8818	-32845	-96911	-19042
0.13	-518	69937	586	455	356	318	335	429	1091	2067	5991	143274	718489	-19521
0.14	-529	3368	486	385	305	273	287	365	892	1671	4450	21097	71229	-20066
0.15	-541	1666	410	331	264	236	249	315	746	1386	3486	11012	36201	-20687
0.16	-554	1081	352	288	231	207	218	274	635	1172	2831	7288	23728	-21393
0.17	-568	787	305	253	203	183	192	241	548	1007	2359	5358	17360	-22201
0.18	-585	611	268	224	181	162	171	214	479	876	2005	4184	13514	-23127
0.19	-603	494	237	200	162	145	153	191	422	770	1730	3397	10949	-24194
0.20	-624	411	212	179	146	131	138	172	376	683	1512	2835	9124	-25431
0.21	-647	350	190	162	132	119	125	155	336	610	1335	2414	7764	-26875
0.22	-674	302	172	147	120	108	113	141	303	549	1189	2090	6714	-28576
0.23	-704	264	156	134	110	99	104	129	275	497	1066	1832	5882	-30604
0.24	-739	234	142	123	100	90	95	118	250	452	963	1623	5207	-33054
0.25	-778	209	130	113	92	83	87	109	229	413	875	1450	4651	-36064
% of Catch	**0.8**	**6.2**	**10.0**	**11.8**	**14.3**	**15.6**	**15.0**	**12.3**	**6.3**	**3.8**	**2.0**	**1.4**	**0.5**	**0.1**

Table 2. A series of sample sizes for ageing bluefish with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6	7	8	9	10	11
0.05	-988	884	-2076	-2256	-1516	-711	-999	-1889	-1939	-1755	-1045	-3369
0.06	-1070	370	2911	-2464	-1599	-766	-1088	-2001	-2011	-1785	-1059	-3404
0.07	-1186	219	758	-2766	-1710	-843	-1216	-2151	-2102	-1821	-1077	-3446
0.08	-1355	149	409	-3221	-1858	-954	-1408	-2354	-2219	-1865	-1098	-3497
0.09	-1616	109	269	-3960	-2060	-1121	-1715	-2636	-2368	-1917	-1122	-3556
0.1	-2059	84	194	-5325	-2346	-1395	-2267	-3044	-2559	-1979	-1151	-3624
0.11	-2956	67	149	-8600	-2770	-1911	-3517	-3673	-2811	-2053	-1185	-3702
0.12	-5649	55	118	-26365	-3454	-3209	-8885	-4746	-3151	-2140	-1225	-3792
0.13	-598355	46	97	21174	-4723	-12288	13479	-6954	-3627	-2243	-1271	-3895
0.14	5328	39	81	7184	-7827	5979	3625	-13982	-4335	-2366	-1324	-4012
0.15	2557	34	69	4202	-26623	2303	2031	163817	-5484	-2515	-1387	-4147
0.16	1643	29	59	2911	16992	1389	1381	11225	-7653	-2696	-1462	-4300
0.17	1191	26	52	2193	6192	977	1030	5636	-13217	-2920	-1550	-4477
0.18	921	23	46	1739	3699	743	812	3689	-57732	-3201	-1656	-4682
0.19	744	20	40	1426	2595	593	663	2702	22546	-3565	-1786	-4919
0.2	618	18	36	1199	1973	489	556	2107	9144	-4050	-1946	-5196
0.21	525	16	33	1027	1577	413	475	1712	5627	-4725	-2148	-5524
0.22	453	15	29	893	1302	355	412	1430	4010	-5727	-2412	-5915
0.23	396	14	27	785	1101	309	362	1220	3083	-7360	-2767	-6388
0.24	351	13	24	698	949	273	321	1057	2483	-10482	-3269	-6971
0.25	313	12	22	625	829	243	287	929	2064	-18790	-4032	-7704
% of catch	**4.8**	**44.2**	**29.6**	**2.8**	**2.4**	**5.6**	**5.1**	**2.2**	**1.4**	**0.7**	**0.9**	**0.3**

Table 3. A series of sample sizes for ageing spadefish with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age. Age 13-20 are not showed in the table due to small percentage of the catch these age groups take and limited width of the page.

CV	0	1	2	3	4	5	6	7	8	9	10	11	12
0.05	-15	-899	-6401	-677	-1101	-1065	-8857	-2247	-2163	-4783	-4278	-3029	-2608
0.06	-15	-1945	2079	-884	-1208	-1168	-11295	-2632	-2532	-5512	-4740	-3337	-2823
0.07	-15	5184	810	-1384	-1366	-1318	-16741	-3300	-3173	-6724	-5433	-3793	-3127
0.08	-16	991	475	-3992	-1608	-1548	-37738	-4668	-4480	-9009	-6537	-4503	-3572
0.09	-16	517	324	3517	-2012	-1929	89542	-8800	-8405	-14654	-8491	-5716	-4257
0.1	-16	337	239	1134	-2797	-2662	18774	-844526	-404919	-48891	-12753	-8176	-5420
0.11	-16	243	185	648	-4920	-4587	10021	8123	7917	30899	-28644	-15595	-7764
0.12	-16	187	148	441	-29110	-22066	6633	3858	3741	11085	78537	-2568472	-14750
0.13	-17	149	122	328	6700	7024	4851	2456	2377	6532	15499	14517	-672406
0.14	-17	122	103	256	2877	2898	3760	1764	1706	4525	8302	6959	14260
0.15	-17	102	87	208	1784	1777	3028	1354	1309	3402	5539	4463	6801
0.16	-17	87	76	173	1269	1257	2507	1085	1048	2689	4086	3226	4362
0.17	-18	75	66	146	970	959	2119	895	865	2198	3194	2491	3157
0.18	-18	66	58	126	777	766	1820	755	729	1842	2593	2006	2441
0.19	-19	58	52	110	641	631	1583	648	626	1572	2163	1664	1969
0.2	-19	52	46	97	542	533	1393	564	544	1362	1842	1410	1636
0.21	-20	46	42	86	466	458	1236	496	479	1194	1592	1216	1389
0.22	-20	42	38	77	406	399	1106	440	425	1058	1395	1062	1199
0.23	-21	38	34	70	358	351	996	394	380	945	1234	938	1049
0.24	-22	35	31	63	319	313	903	355	343	850	1102	836	928
0.25	-23	32	29	57	286	280	822	322	311	769	991	750	828
% of catch	**1.5**	**22.9**	**27.4**	**15.2**	**5.1**	**5.2**	**2.1**	**4.7**	**4.8**	**2.3**	**1.9**	**2.4**	**2.3**

Table 4. A series of sample sizes for ageing Spanish mackerel with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6	7	10
0.01	-202	-275	-1387	-2428	-2097	-1051	-1815	-3920	-5076
0.02	-202	-481	-1573	-2588	-2183	-1066	-1828	-3938	-5083
0.03	-203	1934	-2023	-2905	-2343	-1090	-1850	-3968	-5095
0.04	-203	241	-3377	-3506	-2612	-1127	-1881	-4011	-5112
0.05	-204	113	-24257	-4778	-3063	-1178	-1924	-4068	-5135
0.06	-205	69	3700	-8584	-3883	-1247	-1978	-4140	-5162
0.07	-206	47	1566	-146534	-5680	-1339	-2046	-4227	-5195
0.08	-208	34	941	8352	-12190	-1465	-2131	-4334	-5233
0.09	-209	26	647	3800	40772	-1639	-2236	-4461	-5278
0.1	-211	21	480	2362	6963	-1890	-2367	-4612	-5328
0.11	-213	17	374	1665	3633	-2275	-2530	-4791	-5385
0.12	-215	14	300	1258	2384	-2928	-2737	-5004	-5448
0.13	-218	12	248	994	1736	-4256	-3003	-5258	-5519
0.14	-221	10	208	811	1342	-8344	-3357	-5563	-5598
0.15	-224	9	178	677	1079	263412	-3842	-5933	-5685
0.16	-227	8	154	575	892	7355	-4545	-6386	-5781
0.17	-231	7	135	496	753	3615	-5643	-6952	-5886
0.18	-235	6	119	432	646	2348	-7588	-7674	-6003
0.19	-239	5	106	381	562	1713	-11939	-8619	-6131
0.2	-244	5	95	338	494	1334	-30178	-9905	-6272
0.21	-250	4	85	303	438	1081	49806	-11749	-6428
0.22	-255	4	77	273	392	903	13177	-14598	-6600
0.23	-262	4	70	247	353	769	7446	-19562	-6790
0.24	-269	3	64	225	320	667	5120	-30337	-7000
0.25	-277	3	59	206	291	585	3863	-71256	-7234
% of catch	**0.7**	**65.5**	**17.7**	**6.7**	**5.0**	**3.0**	**1.0**	**0.3**	**0.1**

Table 5. A series of sample sizes for ageing spot with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6
0.05	-4339	251	1692	-1806	-2407	-3050	-4397
0.06	-4704	153	940	-2714	-2620	-3127	-4437
0.07	-5222	105	617	-6683	-2925	-3222	-4485
0.08	-5983	77	441	9724	-3380	-3340	-4542
0.09	-7167	59	334	2571	-4103	-3484	-4608
0.1	-9201	47	262	1411	-5393	-3661	-4685
0.11	-13407	38	212	941	-8263	-3878	-4772
0.12	-26852	32	175	690	-19814	-4148	-4871
0.13	298501	27	148	535	38145	-4487	-4984
0.14	21192	23	126	430	9171	-4922	-5112
0.15	10607	20	109	356	5051	-5494	-5257
0.16	6915	17	95	300	3412	-6273	-5422
0.17	5046	15	84	257	2536	-7387	-5608
0.18	3921	14	74	223	1993	-9103	-5821
0.19	3174	12	67	196	1626	-12066	-6064
0.2	2643	11	60	174	1361	-18366	-6343
0.21	2247	10	54	155	1162	-40719	-6665
0.22	1943	9	49	139	1008	147292	-7041
0.23	1701	8	45	126	885	25256	-7482
0.24	1506	8	41	115	785	13539	-8005
0.25	1345	7	38	105	702	9126	-8635
% of catch	**1.4**	**57.7**	**26.6**	**10.9**	**2.5**	**0.6**	**0.2**

Table 6. A series of sample sizes for ageing spotted seatrout with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6	7	8
0.05	-127	440	-1486	-983	-1207	-1701	-352	-1930	0
0.06	-142	196	-6832	-1100	-1282	-1761	-356	-1937	0
0.07	-164	118	2101	-1282	-1384	-1837	-361	-1945	0
0.08	-199	81	837	-1585	-1523	-1934	-368	-1954	0
0.09	-265	60	498	-2162	-1719	-2056	-375	-1965	0
0.1	-417	46	343	-3649	-2008	-2214	-384	-1977	0
0.11	-1146	37	255	-15188	-2467	-2418	-394	-1991	0
0.12	1251	30	199	6164	-3291	-2689	-405	-2006	0
0.13	382	25	161	2438	-5163	-3063	-419	-2023	0
0.14	218	22	133	1475	-13399	-3604	-434	-2041	0
0.15	150	19	112	1036	18786	-4449	-453	-2061	0
0.16	112	16	96	786	5266	-5935	-474	-2083	0
0.17	88	14	84	625	2981	-9210	-498	-2107	0
0.18	72	13	73	514	2042	-22211	-528	-2133	0
0.19	60	11	65	432	1532	45121	-562	-2161	0
0.2	52	10	58	370	1212	10755	-604	-2192	0
0.21	45	9	52	322	994	5973	-656	-2225	0
0.22	39	8	47	283	837	4073	-721	-2260	0
0.23	35	8	43	251	718	3056	-803	-2299	0
0.24	31	7	39	225	625	2424	-913	-2341	0
0.25	28	6	36	203	550	1994	-1063	-2386	0
% of catch	**14.0**	**50.9**	**22.3**	**6.4**	**3.2**	**1.5**	**1.5**	**0.1**	**0.1**

Table 7. A series of sample sizes for ageing striped bass with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age. Age 2 and 15-19 are not showed in the table due to small percentage of the catch these age groups take and limited width of the page.

CV	3	4	5	6	7	8	9	10	11	12	13	14
0.05	-14288	25880	6310	4287	3184	3197	2406	3159	4850	9409	24039	-55448
0.06	-33654	8096	3628	2694	2054	2135	1603	2055	3092	5571	12622	204668
0.07	55922	4468	2415	1872	1447	1533	1149	1455	2165	3759	8084	31275
0.08	13736	2945	1743	1384	1079	1157	867	1088	1608	2733	5714	15815
0.09	7405	2124	1325	1069	838	905	678	846	1246	2087	4289	10137
0.1	4888	1620	1045	852	670	728	545	678	995	1651	3354	7234
0.11	3553	1283	847	696	549	598	448	556	814	1342	2703	5494
0.12	2735	1045	701	580	458	501	375	464	678	1113	2229	4349
0.13	2187	870	591	490	388	426	318	393	575	939	1872	3546
0.14	1798	736	505	421	333	366	274	338	493	803	1596	2956
0.15	1510	632	437	365	289	318	238	294	428	696	1378	2508
0.16	1289	549	382	319	254	279	209	257	375	608	1202	2158
0.17	1115	482	337	282	224	247	185	227	331	537	1059	1879
0.18	976	426	299	251	200	220	165	203	295	477	939	1653
0.19	862	380	268	225	179	197	148	182	264	427	840	1466
0.2	767	341	241	203	161	178	133	164	238	384	755	1310
0.21	688	308	218	183	146	161	121	148	216	348	683	1178
0.22	621	279	198	167	133	147	110	135	196	316	620	1066
0.23	563	255	181	153	122	134	100	123	179	289	566	969
0.24	514	233	166	140	112	123	92	113	165	265	519	885
0.25	470	214	153	129	103	114	85	104	152	244	478	812
% of catch	**2.8**	**5.8**	**8.3**	**10.0**	**12.2**	**11.7**	**14.7**	**12.2**	**8.8**	**5.7**	**3.1**	**1.8**

Table 8. A series of sample sizes for ageing summer flounder with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6	7	8	9	10
0.05	-7645	2699	1100	1431	2444	8825	-19362	-10235	-10752	-3080	-9352
0.06	-7965	1224	715	937	1529	4244	157377	-14180	-12012	-3104	-9487
0.07	-8380	743	506	666	1060	2631	13351	-26041	-13944	-3133	-9652
0.08	-8916	512	378	499	783	1828	6494	-747484	-17119	-3166	-9849
0.09	-9613	378	294	389	604	1359	4105	24590	-23075	-3205	-10083
0.1	-10533	293	236	312	481	1056	2909	11414	-37758	-3250	-10357
0.11	-11778	234	193	256	393	847	2200	7168	-127233	-3301	-10679
0.12	-13531	192	161	214	327	696	1737	5093	79749	-3359	-11055
0.13	-16142	161	137	181	277	583	1413	3874	28808	-3424	-11495
0.14	-20391	137	118	156	237	496	1176	3079	17048	-3497	-12011
0.15	-28428	118	102	135	206	428	997	2522	11851	-3579	-12619
0.16	-49131	103	89	119	180	373	857	2114	8939	-3671	-13342
0.17	-218565	90	79	105	159	328	746	1803	7085	-3775	-14208
0.18	82240	80	70	93	142	291	656	1560	5808	-3891	-15258
0.19	33500	71	63	84	127	260	581	1365	4878	-4022	-16552
0.2	20619	64	57	75	114	234	519	1206	4174	-4170	-18176
0.21	14684	58	52	68	103	211	467	1075	3624	-4338	-20267
0.22	11279	53	47	62	94	192	422	965	3184	-4529	-23048
0.23	9076	48	43	57	86	175	384	871	2825	-4748	-26913
0.24	7539	44	39	52	79	160	350	791	2527	-5000	-32626
0.25	6407	40	36	48	73	147	321	722	2277	-5293	-41899
% of catch	**0.4**	**18.9**	**26.5**	**22.4**	**16.0**	**8.5**	**4.2**	**2.0**	**0.8**	**0.2**	**0.1**

Table 9. A series of sample sizes for ageing tautog with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age. Age 13-17 and 25 are not showed in the table due to small percentage of the catch these age groups take and limited width of the page.

CV	1	2	3	4	5	6	7	8	9	10	11	12
0.05	-704	-22345	1821	1755	2632	5703	37101	-375992	-42523	-12967	-4414	-12373
0.06	-721	13733	1088	1136	1697	3159	11308	20767	75892	-19463	-4895	-15066
0.07	-742	4722	737	802	1195	2069	6208	9242	17686	-47714	-5618	-20280
0.08	-767	2688	537	599	891	1479	4083	5634	9383	70708	-6774	-33764
0.09	-799	1806	411	465	692	1118	2942	3906	6124	18545	-8833	-137014
0.1	-838	1321	325	372	553	879	2241	2909	4412	10164	-13377	56671
0.11	-885	1019	264	305	453	710	1775	2269	3370	6778	-31014	22116
0.12	-943	815	220	255	378	587	1445	1828	2678	4967	69857	13261
0.13	-1016	669	185	216	320	494	1202	1509	2189	3848	15403	9239
0.14	-1108	561	159	185	275	422	1018	1270	1828	3096	8363	6960
0.15	-1228	478	137	161	239	365	874	1086	1554	2558	5609	5502
0.16	-1388	413	120	141	209	318	759	940	1339	2158	4149	4495
0.17	-1613	360	106	125	185	281	666	822	1167	1850	3248	3763
0.18	-1946	317	94	111	165	249	589	725	1027	1606	2641	3208
0.19	-2490	282	84	99	148	223	525	645	911	1410	2205	2775
0.2	-3531	252	76	90	133	200	471	578	815	1249	1878	2430
0.21	-6298	227	69	81	121	181	425	521	733	1116	1625	2149
0.22	-35363	206	62	74	110	165	386	472	663	1003	1423	1917
0.23	9234	187	57	68	100	150	352	430	603	907	1260	1722
0.24	3985	171	52	62	92	138	322	393	551	825	1125	1556
0.25	2502	157	48	57	85	127	296	361	506	754	1012	1415
% of catch	**1.8**	**8.6**	**23.0**	**21.1**	**15.5**	**10.8**	**5.1**	**4.2**	**3.1**	**2.2**	**1.8**	**1.2**

Table 10. A series of sample sizes for ageing weakfish with CVs ranging from 0.05 to 0.25. The effective sample size is highlighted in blue. The highlighted cells indicate a specific acceptable CV for a particular age when the effective sample size is used to age.

CV	0	1	2	3	4	5	6	7	8	9	10	11
0.05	-2511	-7973	777	1675	6644	-10191	-7604	-6866	-2837	-4602	-1362	-5554

0.06	-2529	12213	448	1043	2500	38054	-10583	-7937	-2920	-4666	-1365	-5564
0.07	-2551	3059	299	722	1439	5770	-19711	-9731	-3024	-4743	-1369	-5576
0.08	-2577	1641	216	532	966	2916	-4125240	-13163	-3154	-4836	-1373	-5589
0.09	-2607	1075	164	410	704	1868	17550	-21932	-3316	-4945	-1378	-5605
0.1	-2642	776	130	327	540	1333	8269	-85844	-3518	-5074	-1384	-5622
0.11	-2681	594	105	266	430	1013	5219	38653	-3772	-5223	-1390	-5642
0.12	-2725	472	87	222	351	801	3717	14933	-4096	-5398	-1397	-5663
0.13	-2774	386	73	188	293	653	2831	8958	-4517	-5602	-1404	-5687
0.14	-2830	323	63	161	248	545	2252	6255	-5081	-5840	-1412	-5712
0.15	-2892	274	54	139	213	462	1846	4724	-5868	-6119	-1421	-5740
0.16	-2962	237	47	122	186	398	1548	3744	-7034	-6448	-1431	-5770
0.17	-3040	206	42	108	163	346	1321	3067	-8919	-6840	-1441	-5802
0.18	-3128	182	37	96	144	305	1143	2574	-12462	-7311	-1452	-5837
0.19	-3226	161	33	86	129	270	1001	2199	-21481	-7886	-1464	-5874
0.2	-3336	144	30	77	116	241	884	1907	-90617	-8598	-1477	-5913
0.21	-3460	130	27	70	104	217	788	1673	38020	-9500	-1491	-5955
0.22	-3601	117	25	64	95	196	707	1483	15276	-10674	-1506	-6000
0.23	-3761	107	22	58	86	179	639	1325	9395	-12259	-1522	-6048
0.24	-3944	97	21	53	79	163	580	1192	6700	-14511	-1538	-6098
0.25	-4155	89	19	49	73	150	530	1080	5158	-17948	-1556	-6152
% of catch	**0.2**	**12.1**	**36.0**	**22.4**	**15.3**	**8.4**	**2.9**	**1.6**	**0.7**	**0.2**	**0.1**	**0.0**

Chapter 15

The role of ageing error in backward age-structured stock assessment analysis, a case study of the ADAPT-VPA stock assessment of the striped bass, *Morone saxatilis*, in Mid and North Atlantic Coasts of the United States

Introduction

Age-structured stock assessment analysis has been developed in a variety of computer models for several decades. In terms of procedures of parameter estimation, there are two main scenarios: backward and forward projection models. The backward projection calculates parameters starting with the oldest ages backward to the youngest ages through each cohort whereas the forward projection does this in an opposite direction (Megrey 1989). Under each of these scenarios, several models have been developed, and some of them are used more widely than others.

The ADAPT-VPA is one of the backward projection models (Gavaris 1988) and has been used in stock assessment for many marine fish species (Myers et al. 1997; Hiramatsu and Tanaka 2004). However, previous studies indicated that ADAPT-VPA was very sensitive to its assumptions. For example, Hiramatsu and Tanaka (2004) found that ADAPT-VPA would not give reliable estimates of population abundance when fishing mortalities remained constant for all years and cumulative fishing mortalities were similar among cohorts.

One important assumption for the ADAPT-VPA is that ageing is free of error. Ageing errors have been reported to affect estimates of recruitment, growth, and mortality in many fish species (Lai and Gunderson 1987; Bradford 1991; de Pontual et al. 2006). However, little is known about the impact of ageing errors in ADAPT-VPA stock assessment. Such information is important because many fisheries management decisions are made based on ADAPT-VPA analysis along the U.S. East Coast. Understanding of the influence of ageing error on ADAPT-VPA analysis will improve our decision-making. To do so, we used the striped bass (*Morone saxatilis*) fishery along the Mid and North Atlantic coasts as a case study.

Striped bass is one of the most important species in terms of recreational and commercial fisheries along the Mid and North Atlantic coasts. Striped bass have successfully recovered from a depleted population during the early 1980s to a fully restored fishery in recent years. To understand the population dynamics of striped bass in Mid and North Atlantic and assist its fishery management, Atlantic States Marine Fisheries Commission (ASMFC 2005) started to conduct its assessment using ADAPT-VPA in 1996. The

assessment relied on scales for age information. However, it has been well documented that ageing fish with scales (including striped bass) is problematic especially when fish are older because scale growth slows considerably with age (Beamish and McFarlane 1987). For example, Welch et al. (1993) reported that it was difficult to discerning annuli on scales of larger striped bass because of crowding near the margins. As a result, there are measurement errors (ageing errors) in scale-ages of striped bass. Whereas, previous studies have indicated that otoliths are superior to scales to age for many fish species, including striped bass (Erickson 1983; Libby 1985; Boxrucker 1981; Barber and McFarlane 1987; Heidinger and Clodfelter 1987; Welch et al. 1993; Lowerre-Barbieri et a. 1994; Secor et al. 1995; Brown et al. 2004; Brouder 2005; Decicco and Brown 2006). Moreover, otolith-based ageing of striped bass was validated using known-age fish (Secor et al. 1995). Secor et al. (1995) reported that otolith-ages provided more accurate and precise age estimates of striped bass up to age 31 compared to scale ages.

Theoretically and ideally, true-age input data should be used to examine the role of ageing errors induced by scale ages in ADAPT-VPA stock assessment. However, there are no true-age data containing many cohorts for many years. For example, ASMFC in 2003 (personal communication) held an ageing workshop which used scales to age known-age striped bass and found that the scale ages were very similar to the true age for fish younger than 10 years old. Unfortunately, they didn't have sufficient samples to examine differences between scale and known ages for fish older than age 10 (4 out of 102 fish were older than age 10). Therefore, although otolith age is neither a true age nor free of error, it becomes the best choice by assuming otolith age as true age to examine the role of ageing error induced by scale-ageing error in ADAPT-VPA stock assessment.

In this study, we compared population parameters of striped bass estimated using both scale and otolith-based data in ADAPT-VPA analysis. Our objectives were to: 1) compare catch-at-age data derived from both scale and otolith ages; 2) examine population abundance, spawning stock biomass, fishing mortality, and recruitment estimated from the ADAPT-VPA using both scale and otolith-based data; 3) explore influence of ageing errors induced by scale ages on performance of the ADAPT-VPA analysis.

Methods

1. *Data collection*

Striped bass were collected by Virginia Marine Resources Commission (VMRC) in Virginia waters of the Chesapeake Bay and Atlantic from 1999 to 2004. Fish were measured to 1 mm and weighed to 0.454 g (converted from 0.001 pound). Both scales and otoliths were removed from each fish. For age determination, scales were impressed on acetate slides using a hydraulic heated press, and otoliths were thin-sectioned using a low speed saw and baked in a Thermolyne 1400 furnace at 400°C.

2. *Ageing*

The acetate impressions of scales were viewed with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Sectioned otoliths were aged using a Leica MZ-12 dissecting microscope with transmitted light and dark-field polarization at between 8 and 100 times magnification. Two readers aged all samples separately 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 two readers were unable to agree on a final age, the fish was excluded from further analysis.

3. *Conversion of scale-base ADAPT input data*

ASMFC provided all scale-based age ADAPT-VPA input data from 1982 to 2004, including catch at age (CAA), tuning indices, and weight at age (WAA). These input data were converted to otolith-based input data using conversion matrices developed in this study.

1) Conversion matrices
A conversion matrix is a frequency table of the otolith age of a fish when it is aged using scales. Two kinds of conversion matrices were developed, year-specific and cumulative conversion matrices. Each year-specific conversion matrix was developed from striped bass with both scale and otolith ages obtained in Virginia waters in each year during the period from 1999 to 2004, and the cumulative conversion matrix was developed from all fish with both scale and otolith ages pooled from 1999 to 2004.

2) Conversion of catch at age (CAA)
Each year-specific conversion matrix was applied to each year's CAA for the period of 1999 and 2004. For the period of 1982 to 1998, when we didn't have paired scale and otolith ages we used the cumulative conversion matrix to convert scale-based CAA to otolith-based CAAs.

3) Conversion of tuning indices
Scale-based tuning indices were also converted to otolith-based ones for the period of 1982 and 1998 and the period of 1999 and 2004 using the cumulative and year-specific matrices, respectively.

4) Weight at age (WAA)

Otolith-based WAA were calculated directly from fish collected in Virginia waters from 1999 to 2004. Because we didn't have weight-at-otolith-age in Virginia from 1982-1998, we used the mean weight-at-otolith-age derived from 1999 to 2004 to replace the weight-at-scale age for the coast from 1982 to 1998. Specifically, we calculated the mean weight-at-otolith age and –scale-age using pooled Virginia data from 1999 to 2004. Subsequently we used the following equation to calculate year-specific mean weight-at-otolith-age for the coast from 1982 to 1998:

$$CWAA_{oto,\,y} = CWAA_{scl,y} \bullet \frac{VWAA_{oto}}{VWAA_{scl}} \qquad (1)$$

Where CWAA and VWAA represent the year-specific coast and pooled Virginia weight at age, respectively. Subscripts of oto, scl, and y represent the otolith and scale age, and year, respectively.

4. *ADAPT analysis*

Two parallel ADAPT-VPA runs were conducted. One was based on the input data derived from scale ages provided by ASMFC and hereafter called the base run, another was based on the input data converted from otolith ages and hereafter called the corrected run. The rest of the model configurations were the same between two parallel runs set up by the Striped Bass Stock Assessment Subcommittee of ASMFC. Four outputs compared between two runs were population abundance (N), spawning stock biomass (SSB), fishing mortality (F), and recruitment (R),.

5. *Data analysis*

1) Comparison between scale and otolith ages

We used a symmetry test (Hoenig et al. 1995) to detect any systematic difference between scale and otolith ages collected from Virginia waters between 1999 and 2004.

2) Comparison between scale-based and otolith-based CAAs

The Mantel-Haenszel chi-square test was used to examine difference between scale- and otolith-based CAAs (using SAS).

3) Comparisons of abundance, spawning stock biomass, and fishing mortality between the base and corrected run of ADAPT-VPA analysis.

To compare the outputs from the base and corrected runs, we fitted the 6-order polynomial regression models to the output data from each of two runs, respectively. The full model (M_{full}) was:

$$\text{Log}(y) = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + b_5x^4 + b_6x^6,$$

where y represents N, SSB, and F, respectively, and x represents year.

The AIC_c, instead of AIC, was used to select the best models because the ratio of data points to the number of parameters was smaller than 40 (Burnham and Anderson 1998). AIC_c was calculated using the following equation:

$$AIC_c = AIC + \frac{2P(P+1)}{(X-P-1)},$$

where P and X are the number of parameters and the number of years in the model, respectively (Burnham and Anderson 1998).

The criteria for model selection using AIC_c are as follows:
i) When the best-fit polynomial regressions were in the same order for both base and corrected run, these best-fit models were selected for further comparison between the outputs from the base and corrected run;

ii) When the best-fit polynomial regressions were in different orders for the base and corrected run, we selected the same order models for both runs if the difference (Δi) in AIC_c values between the same order model selected and the best-fit model for each run was smaller than 10 (Burnham and Anderson 1998).

iii) Once the same order models for both runs were selected, two models were compared by introducing a dummy variable for intercept.

We compared two models between the base and corrected run using a maximum likelihood method with autoregression error structure. This allowed us to eliminate autoregression effects due to year dependence in the outputs of ADAPT. Including autoregression error structure would improve model fitting with an increased R^2, a decreased AIC value, and a Durbin-Watson (DW) value closer to 2. The outputs between two runs were considered to be different when the intercepts between two models were significantly different.

4) We examined the patterns of recruitments between the base and corrected run between 1982 and 2004 by tracking strong cohorts through time.

5) We conducted retrospective analysis of fishing mortality for age 8 to 11 for four years from 2003 back to 2000. Next, we examined the retrospective patterns using the following methods. First, we calculated the differences between two fishing mortalities for a previous year estimated from the previous modeling year and from the current modeling year within each run, respectively. For example, the fishing mortality in 2003 estimated in 2003 minus the fishing mortality in 2003 estimated in 2004 in the base and corrected run, respectively. And then, we compared the two sets of such differences derived from the based and corrected run using a paired t-test.

Results

1. Comparison between scale and otolith ages

The symmetry test indicated that there was a systematic disagreement between scale and otolith ages ($X^2 = 272.05$, $df = 72$, and $P < 0.0001$) and mean coefficient of variance is 7.8% (Figure 1). In general, the scale age overestimated age of younger fish whereas they underestimated age of older fish compared to the otolith age. The average coefficients of variation for the otolith were smaller than those for the scales (Table 1).

2. Comparison between scale-based and otolith-based CAAs

Mantel-Haenszel chi-square test indicated that there were very strong associations between CAA and ageing methods within each sampling year and for all sampling years combined ($X^2 = 67{,}885$, df = 11, and $P < 0.0001$). It is much easier to track a strong cohort through the years using otolith-based CAA than using scale-based CAA because scale-based CAA is smoother (spreads error to adjacent years) through years compared to otolith-based CAA (Figure 2).

3. Outputs of the ADAPT-VPA analysis

By including autoregression error structure, the model fitting was significantly improved for all three comparisons, abundance, spawning stock biomass, and fishing mortality. Compared to ordinary least square analysis, maximum likelihood analysis with autoregression error structure increased R^2, decreased AIC, and made Durbin-Watson (DW) values closer to 2 (DW value of 2 indicates no autoregression errors) (Table2).

1) Abundance (N)

AIC_c selected M_{full} and M_{1234} as the best-fit models for the base and corrected run, respectively (Table 3). To compare the outputs of abundance between the base and corrected run, we selected M_{12345} for both runs because the values of Δi between M_{12345} and M_{full} for the base run and between M_{12345} and M_{1234} for the corrected run were 7.8 and 3.0, respectively (Table 3). The intercepts were significantly different between the two models (Table 6), indicating that different population abundance was estimated by the base and corrected run (Figure 3).

2) Spawning stock biomass (SSB)
AIC_c selected M_{12346} and M_{1245} as the best-fit models for the base and corrected run, respectively (Table 4). To compare the outputs of spawning stock biomass between the base and corrected run, we selected M_{1234} for both runs because the values of Δi between M_{1234} and M_{12346} for the base run and between M_{1234} and M_{1245} for the corrected run were 5.6 and 9.8, respectively (Table 4). The intercepts were significantly different between two models (Table 6), indicating that different spawning stock biomass was estimated by the base and corrected run (Figure 4).

3) Fishing mortality (F)
AIC_c selected M_{136} and M_{134} as the best-fit models for the base and corrected run, respectively (Table 5). To compare the outputs of fishing mortality between the base and corrected run, we selected M_{136} for the corrected run because the values of Δi between M_{136} and M_{134} for the corrected run were 1.6 (Table 5). The intercepts were significantly different between two models (Table 6), indicating that different fishing mortality for Age 8-11 was estimated by the base and corrected run (Figure 5).

4) Recruitment
There were three patterns of recruitment within each run and between two runs through time (Figure 6). From 1982 to 1993, the recruitment was very similar between the two runs. From 1994 to 1999, the recruitment from the base run was flat whereas it fluctuated for the corrected run over the years, resulting in different patterns between the two runs. From 2000 to 2004, the recruitment from both runs varied similarly through the years in terms of pattern and magnitude. In conclusion, the base and corrected run provided different recruitment pattern of striped bass.

5) Retrospective pattern
The retrospective patterns of fishing mortality for age 8 to 11 were different between two runs in terms of pattern and magnitude (Figure 7). In the base run, the previous year F estimated from the previous modeling year was always higher than the previous year F estimated from the current modeling year. However, in the corrected run, the pattern became opposite, that is, the previous year F estimated from the previous modeling year was always lower than the previous year F estimated from the current modeling year. The degree of the retrospective change in the corrected run was smaller than in the base run, too. The paired t-test indicated that there is a significant difference in the retrospective changes between two runs (Table 4).

Discussion

This study is the first to examine how ageing errors influence backward age-structured stock assessment analysis using the multi-year data collected for striped bass along the

Mid and North Atlantic coasts of the US. Although otolith ages are not true ages, the previous studies and our study support that there are fewer ageing errors with otoliths than with scales. Secor et al. (1995) verified that otoliths provided more accurate estimates of age for striped bass ranging in age from 3 to 7 years old by using known-age fish. By assuming otolith ages as true ages, we were able to examine the hypothesis that the ageing errors significantly influence backward age-structured stock assessment analysis using the ADAPT-VPA stock assessment of striped bass in Atlantic as a case study.

The results from this study are consistent with previous studies on the relationship between striped bass scale and otolith ages. For example, Welch et al. (1993) reported that age estimates were lower from scales of striped bass larger than 900 mm than from otoliths by averages of 1.6 and 3.0 years. Secor et al (1995) found that scale ageing underestimated striped bass up to 9 years for age 22-31. In this study, the younger and older fish ages were overestimated and underestimated, respectively when scales were used to age. Therefore, an ageing technique should be validated before a hard part is used to age for the backward age-structured models.

Ageing error influences the backward age-structured stock assessment analysis mainly through catch at age data. Megrey (1989) noted that all of the age-structured stock assessment models are developed mathematically from two basic equations, Baranov's (1918) catch equation and Lotka's (1925) exponential survival model. As our study indicated, estimating fewer younger and more older fish in the otolith based CAAs would provide higher estimates of population abundance and spawning stock biomass, and lower estimates of fishing mortalities in the ADAPT-VPA analysis.

Bradford (1991) found that ageing errors influenced recruitment in sequential population analysis using simulated data. Lapointe et al. (1992) reported that natural mortality could influence recruitments estimated by VPA. Our study with the striped bass data confirmed that recruitment was influenced dramatically by ageing errors in ADAPT-VPA analysis. The degree of influence increased with the number of years between the final modeling year (2005 in this study) and the year when the recruitment was estimated. This is because the ageing errors are accumulated through years within each cohort during the backward projection. Therefore, ageing errors had the least influence on recruitment for current years, between 2000 and 2005.

Cadigan and Farrel (2005) identified that the relationship between abundance indices and stock sizes was a major source of error resulting in retrospective misspecification in sequential population analysis (SPA). However, this study indicated that ageing error could also result in a significant retrospective problem in the backward age-structured stock assessment model. Specifically in the ADAPT-VPA analysis, ageing errors caused overestimation of the current year's fishing mortality – of most interest to fishery

management. This study illustrated that this retrospective problem could be minimized by increasing accuracy of ageing.

The results in our corrected run were similar to those in Welsh et al. (2007). Welsh et al. (2007) suggested using different methods to estimate population parameters for a species of interest so that estimates from different methods could be compared. We compared our estimates of fishing mortality with those in Welsh et al. (2007) which used tag-recapture methods. Welsh et al. (2007) estimated a 0.302 unadjusted and a 0.26 adjusted fishing mortality for Atlantic coastal striped bass equal to and larger than 711 mm (approximately equal to age 9 and older fish in this study) in 2004 (the terminal year in this study). In this study, the corrected run estimated a fishing mortality of 0.301 and a number-weighted fishing mortality of 0.281 for age 8-11 in 2004 whereas the base run provided higher fishing mortalities (a fishing mortality of 0.401 and a number-weighted fishing mortality of 0.361).

To run the parallel ADAPT-VPA in this study, we assumed that the relationship between otolith and scale age in Virginia data is the same as in the coast data and over the period 1982-1998 assuming an average of the period 1999-2004. We are not clear how the violation of these assumptions could influence the output of ADAPT-VPA analysis. We are continuing to collect, process, and age both scales and otoliths of striped bass and expect to run the same parallel ADAPT-VPA analysis without these assumptions in a few years when we have accumulated enough data.

This study is a cooperative effort between Old Dominion University and Maryland Department of Natural Resources (MD DNR). Dr. Alexei Sharov at MD DNR) helped initiating the study and since then he has been working with us closely. In the coming year we will age known-age striped bass (code-wire tagged and recaptured by MD DNR during the past 20 years) using both their scales and otoliths. We believe that comparing scale and otolith ages directly to true age fish will help us further to understand how ageing errors influence the backward age-structure stock assessment models and how we could improve their performance.

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Table 1. The average coefficients of variation for striped bass otolith and scale ages from 1999 to 2004.

Year	Scale %	Otolith %
1999	7.8	2.1
2000	5.7	1.7
2001	4.4	2.4
2002	4.3	1.5
2003	4.0	1.5
2004	5.4	1.7
Mean	5.3	1.8

Table 2. Fitness was improved by including autoregression error structure. OLS stands for ordinary least squares estimates. ML and AR stand for maximum likelihood estimates and autoregression error structure, respectively. DW stands for Durbin-Watson value. DW equals to 2, indicating no autoregression effect; the farther the DW is from 2, the more effect the autoregression error has. N, SSB, and F represent the abundance, spawning stock biomass, and fishing mortality estimates from the ADAPT runs, respectively.

	OLS			ML with AR		
	DW	**AIC_c**	**R^2**	**DW**	**AIC_c**	**R^2**
N	1.7824	-105.6	0.9936	1.8983	-103.8	0.9936
SSB	1.3475	-61.7	0.9919	1.7512	-64.4	0.9927
F	3.1486	6.4	0.7551	2.6730	-10.9	0.8402

Table 3. The first 11 polynomial models with the smallest AIC_c values for the abundance of striped bass from 1982 to 2004 projected by the ADAPT-VPA runs. P stands for the number of parameters in each model. Δi is the difference in AICc values between Model i and the best-fit model.

Model	P	b_0	b_1	b_2	b_3	b_4	b_5	b_6	R^2	AIC	AIC_c	Δi
						Base run						
M_{full}	7	10.620	1.865	-2.295	-1.578	3.052	1.234	-1.771	0.9978	-141.4	-134.4	0.0
M_{12345}	6	10.585	1.802	-1.545	-1.186	0.727	0.791		0.9965	-131.5	-126.6	7.8
M_{1234}	5	10.593	1.623	-1.653	-0.331	0.891			0.9953	-126.7	-123.4	11.0
M_{12346}	6	10.609	1.608	-1.991	-0.296	1.898		-0.738	0.9956	-126.3	-121.4	13.0
M_{12356}	6	10.567	1.797	-1.255	-1.157		0.758	0.466	0.9955	-126.1	-121.1	13.3
M_{1236}	5	10.574	1.630	-1.319	-0.349			0.601	0.9946	-123.2	-119.9	14.5
M_{1245}	5	10.593	1.530	-1.662		0.904	-0.248		0.9943	-122.3	-118.9	15.5
M_{12456}	6	10.609	1.519	-1.992		1.903	-0.206	-0.742	0.9946	-121.5	-116.5	17.8
M_{1256}	5	10.575	1.536	-1.327			-0.269	0.618	0.9936	-119.4	-116.1	18.3
M_{1246}	5	10.616	1.442	-2.181		2.619		-1.383	0.9934	-118.6	-115.3	19.1
M_{124}	4	10.584	1.432	-1.522		0.690			0.9922	-116.6	-114.5	19.9
						Corrected run						
M_{1234}	5	10.759	1.524	-1.749	-0.326	1.006			0.9925	-118.6	-115.2	0.0
M_{1245}	5	10.761	1.443	-1.771		1.041	-0.269		0.9920	-116.9	-113.6	1.6
M_{12346}	6	10.775	1.509	-2.078	-0.291	1.989		-0.720	0.9929	-117.6	-112.7	2.5
M_{1236}	5	10.738	1.533	-1.374	-0.347			0.683	0.9916	-115.7	-112.3	2.9
M_{12345}	6	10.756	1.591	-1.708	-0.648	0.944	0.298		0.9927	-117.2	-112.2	3.0
M_{1256}	5	10.740	1.450	-1.392			-0.297	0.721	0.9912	-114.4	-111.1	4.1
M_{full}	7	10.780	1.636	-2.227	-0.920	2.555	0.605	-1.227	0.9935	-117.8	-110.8	4.5
M_{12456}	6	10.774	1.434	-2.051		1.885	-0.235	-0.627	0.9922	-115.6	-110.7	4.6
M_{124}	4	10.750	1.336	-1.619		0.808			0.9892	-111.6	-109.5	5.7
M_{1246}	5	10.782	1.346	-2.265		2.699		-1.355	0.9905	-112.7	-109.3	5.9
M_{12356}	6	10.736	1.578	-1.357	-0.567		0.207	0.646	0.9917	-113.9	-109.0	6.3

Table 4. The first 11 polynomial models with the smallest AIC_c values for the spawning stock biomass of striped bass from 1982 to 2004 projected by the ADAPT-VPA runs. P stands for the number of parameters in each model. Δi is the difference in AICc values between Model i and the best-fit model.

Model	P	b_0	b_1	b_2	b_3	b_4	b_5	b_6	R^2	AIC	AIC_c	Δi
						Base run						
M_{12346}	6	9.928	2.743	-3.751	-1.315	7.180		-4.100	0.994	-98.8	-93.5	0.0
M_{full}	7	9.931	2.813	-3.841	-1.701	7.553	0.406	-4.465	0.994	-97.1	-89.6	3.9
M_{12456}	6	9.920	2.473	-3.518		6.217	-1.292	-3.158	0.992	-93.9	-88.6	4.9
M_{1234}	5	9.858	2.813	-2.168	-1.496	2.039			0.991	-91.5	-88.0	5.6
M_{1245}	5	9.869	2.512	-2.335		2.316	-1.469		0.991	-91.3	-87.8	5.7
M_{12345}	6	9.863	2.678	-2.257	-0.794	2.187	-0.710		0.991	-90.3	-85.1	8.4
M_{1256}	5	9.825	2.522	-1.524			-1.516	1.684	0.988	-86.0	-82.5	11.0
M_{1236}	5	9.815	2.820	-1.415	-1.514			1.411	0.988	-85.6	-82.1	11.4
M_{12356}	6	9.821	2.658	-1.483	-0.656		-0.880	1.581	0.988	-84.5	-79.3	14.2
M_{123}	4	9.716	2.681	-0.597	-1.156				0.978	-74.0	-71.7	21.8
M_{1456}	5	9.720	2.533			-3.808	-1.564	4.227	0.981	-74.6	-71.1	22.4
						Corrected run						
M_{1245}	5	10.228	2.524	-2.266		1.866	-1.311		0.997	-115.7	-112.1	0.0
M_{12456}	6	10.245	2.511	-2.654		3.148	-1.253	-1.037	0.997	-115.0	-109.7	2.4
M_{12345}	6	10.230	2.448	-2.301	0.364	1.926	-1.659		0.997	-114.3	-109.0	3.1
M_{1256}	5	10.197	2.536	-1.645			-1.366	1.414	0.996	-110.2	-106.7	5.5
M_{full}	7	10.244	2.475	-2.620	0.182	3.005	-1.434	-0.898	0.997	-113.1	-105.7	6.5
M_{12356}	6	10.200	2.413	-1.682	0.597		-1.946	1.507	0.996	-109.4	-104.2	8.0
M_{12346}	6	10.254	2.725	-2.939	-1.181	4.324		-2.188	0.996	-108.3	-103.0	9.1
M_{1234}	5	10.217	2.762	-2.094	-1.278	1.580			0.995	-105.8	-102.3	9.8
M_{1236}	5	10.187	2.771	-1.532	-1.300			1.130	0.994	-100.8	-97.3	14.9
M_{1456}	5	10.094	2.557			-4.416	-1.458	4.534	0.991	-90.4	-86.9	25.2
M_{123}	4	10.107	2.660	-0.877	-1.014				0.988	-86.8	-84.6	27.6

Table 5. The first 11 polynomial models with the smallest AIC_c values for the fishing mortality of striped bass from 1982 to 2004 projected by the ADAPT-VPA runs. P stands for the number of parameters in each model. Δi is the difference in AICc values between Model i and the best-fit model.

Model	P	b_0	b_1	b_2	b_3	b_4	b_5	b_6	R^2	AIC	AIC_c	Δi
						Based run						
M_{136}	4	-1.711	0.995		-1.761			1.659	0.658	-59.1	-56.9	0.0
M_{156}	4	-1.718	0.619				-1.661	1.776	0.636	-57.7	-55.4	1.4
M_{1346}	5	-1.655	1.058		-1.923	-1.562		3.547	0.685	-58.9	-55.4	1.4
M_{1236}	5	-1.632	1.040	-0.497	-1.878			2.333	0.681	-58.7	-55.2	1.7
M_{1456}	5	-1.645	0.679			-2.077	-1.932	4.325	0.680	-58.6	-55.1	1.8
M_{1256}	5	-1.621	0.659	-0.621			-1.844	2.644	0.671	-58.0	-54.4	2.4
M_{1234}	5	-1.597	0.995	-1.342	-1.762	2.853			0.663	-57.4	-53.9	3.0
M_{1356}	5	-1.710	1.050		-2.046		0.287	1.633	0.659	-57.1	-53.6	3.3
M_{134}	4	-1.743	0.915		-1.556	1.261			0.594	-55.2	-52.9	3.9
M_{1245}	5	-1.587	0.620	-1.507		3.128	-1.669		0.641	-56.0	-52.4	4.4
M_{13456}	6	-1.651	0.925		-1.211	-1.781	-0.740	3.877	0.687	-57.1	-51.9	5.0
						Corrected run						
M_{134}	4	-1.848	0.856		-1.646	1.468			0.814	-76.3	-74.1	0.0
M_{136}	4	-1.797	0.919		-1.808			1.791	0.800	-74.6	-72.4	1.6
M_{1345}	5	-1.841	1.124		-3.014	1.387	1.357		0.827	-75.9	-72.4	1.7
M_{1236}	5	-1.861	0.882	0.406	-1.712			1.241	0.818	-74.8	-71.3	2.8
M_{1346}	5	-1.837	0.874		-1.692	1.112		0.447	0.816	-74.5	-71.0	3.1
M_{1234}	5	-1.841	0.860	-0.064	-1.655	1.544			0.814	-74.3	-70.8	3.3
M_{145}	4	-1.853	0.479			1.523	-1.469		0.768	-71.2	-69.0	5.1
M_{156}	4	-1.803	0.528				-1.690	1.905	0.768	-71.2	-68.9	5.1
M_{1356}	5	-1.794	1.029		-2.379		0.575	1.740	0.802	-72.9	-69.4	4.7
M_{12345}	6	-1.853	1.135	0.117	-3.089	1.242	1.449		0.827	-74.0	-68.7	5.3
M_{13456}	6	-1.845	1.137		-3.103	1.544	1.466	-0.206	0.827	-74.0	-68.7	5.4

Table 6. Comparisons of parameters between the base and corrected runs. P-values smaller than 0.05 indicate that paramters between two models are significantly different. Parameters and their *P*-values were estimated by using maximum likelihood method with autoregression error structure included. N, SSB, and F represent the abundance, spawning stock biomass, and fishing mortality estimates from the ADAPT runs, respectively.

Parameter	Estimate	*P*-value
	N (M_{12345})	
b_0	10.747	< 0.0001
b_1	1.695	< 0.0001
b_2	-1.613	< 0.0001
b_3	-0.913	0.0102
b_4	0.817	< 0.0001
b_5	0.544	0.0808
Dummy	-0.1540	< 0.0001
	SSB (M_{1234})	
b_0	10.179	< 0.0001
b_1	2.800	< 0.0001
b_2	-2.008	< 0.0001
b_3	-1.405	< 0.0001
b_4	1.639	< 0.0001
Dummy	-0.3030	< 0.0001
	F (M_{136})	
b_0	-1.86	< 0.0001
b_1	1.18	< 0.0001
b_3	0.27	0.7416
b_6	-3.39	0.0032
Dummy	0.0739	0.0955

Table 7. Retrospective analysis of fishing mortality for age 8 to 11 estimated from the base and corrected run. * indicates that the values were omitted from the table for visualization because they were not used in the analysis.

	Estimated year			
	2000	2001	2002	2003
Modeling year		**Base run**		
2000	0.3087			
2001	0.2764	0.3194		
2002	*	0.227	0.3454	
2003	*	*	0.2674	0.2852
2004	*	*	*	0.2752
Difference	0.0323	0.0924	0.078	0.01
		Corrected run		
2000	0.211			
2001	0.1933	0.1961		
2002	*	0.2027	0.2096	
2003	*	*	0.2194	0.2265
2004	*	*	*	0.2728
Difference	0.0177	-0.0066	-0.0098	-0.0463

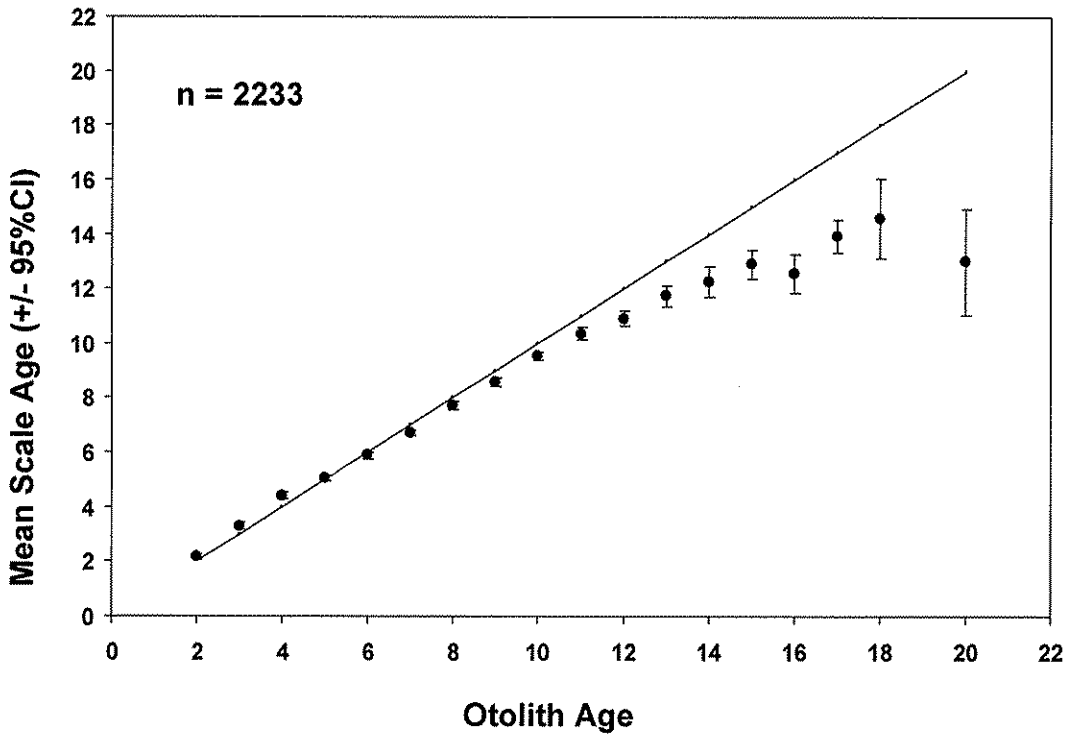


Figure 1. Comparison between scale and otolith ages of striped bass collected from 1999 to 2004. n is number of fish aged. The straight line is one to one line.

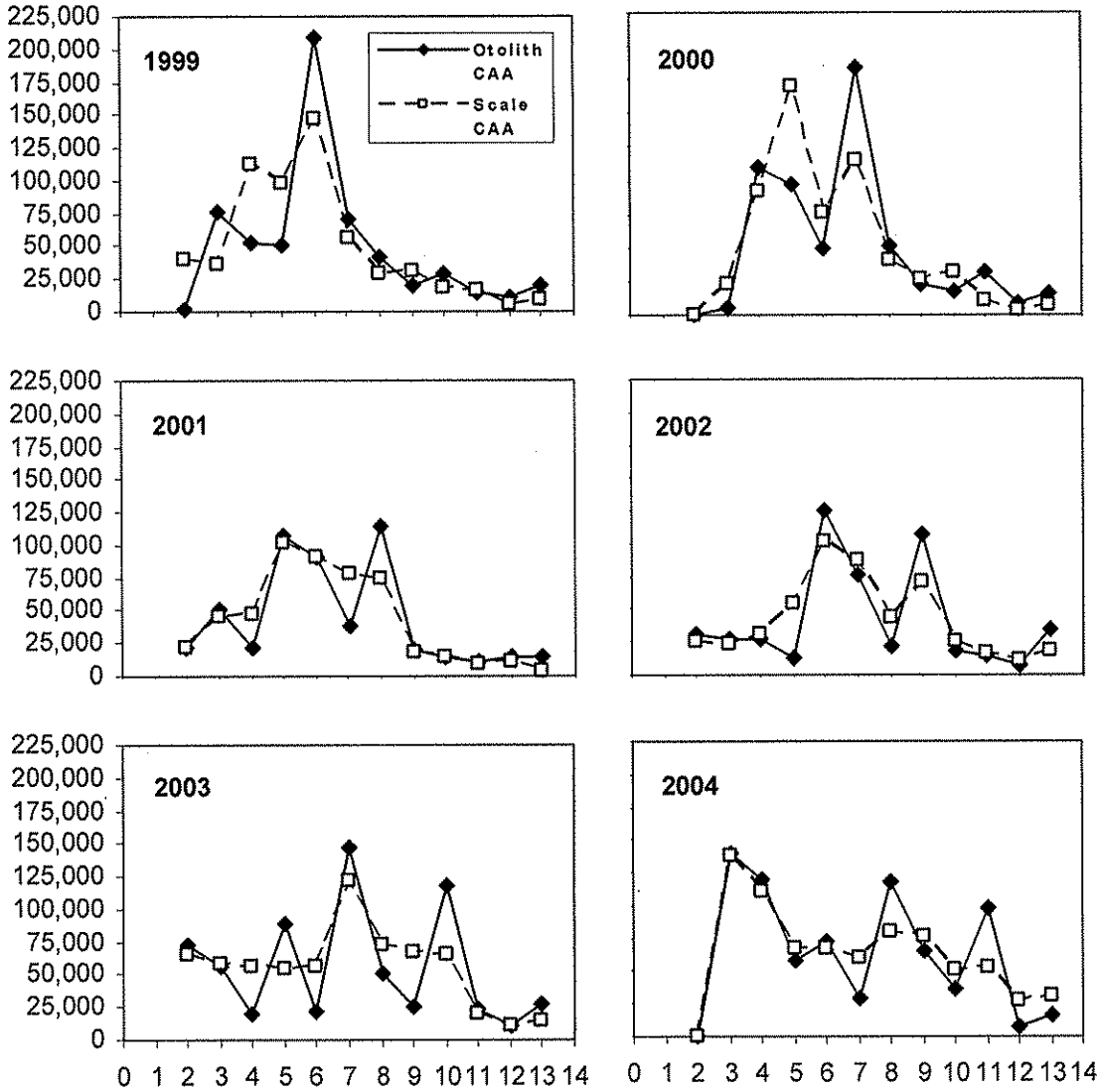


Figure 2. Comparison of Virginia CAA derived from the scale and otolith ages from 1999 to 2004.

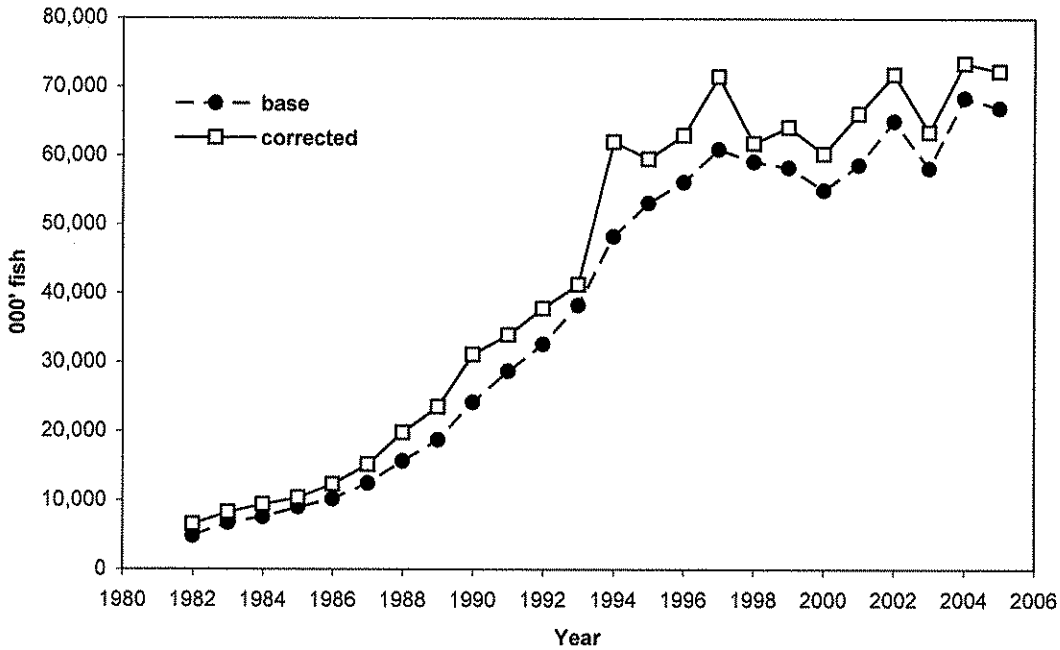


Figure 3. Estimates of fish population abundance on January 1st from the base and corrected run of ADAPT analysis.

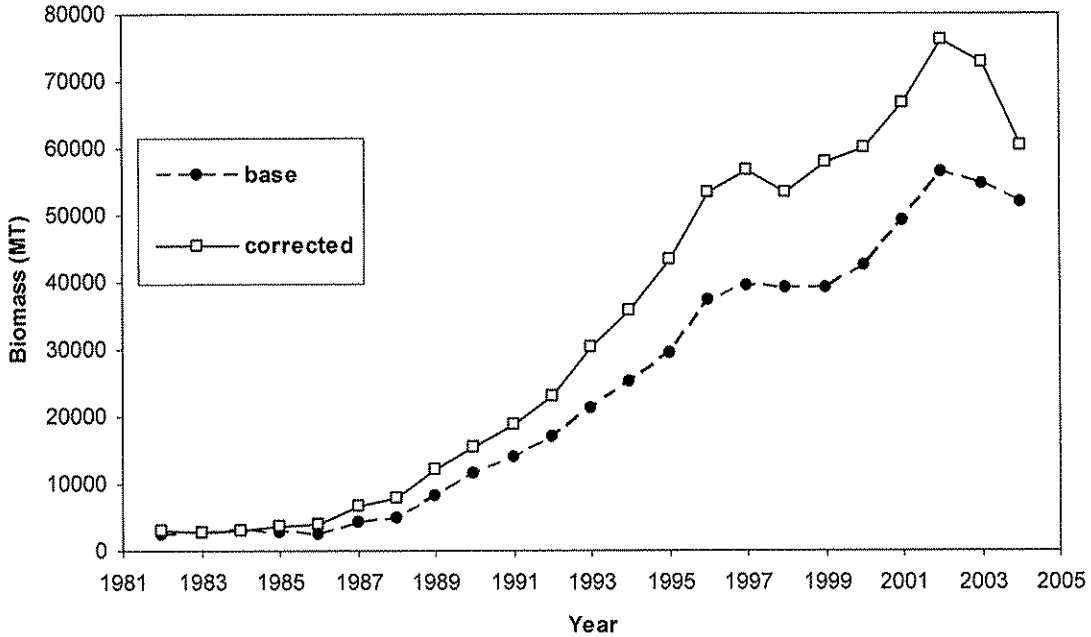


Figure 4. Estimates of spawning stock biomass from the base and corrected run of ADAPT analysis. MT stands for metric ton.

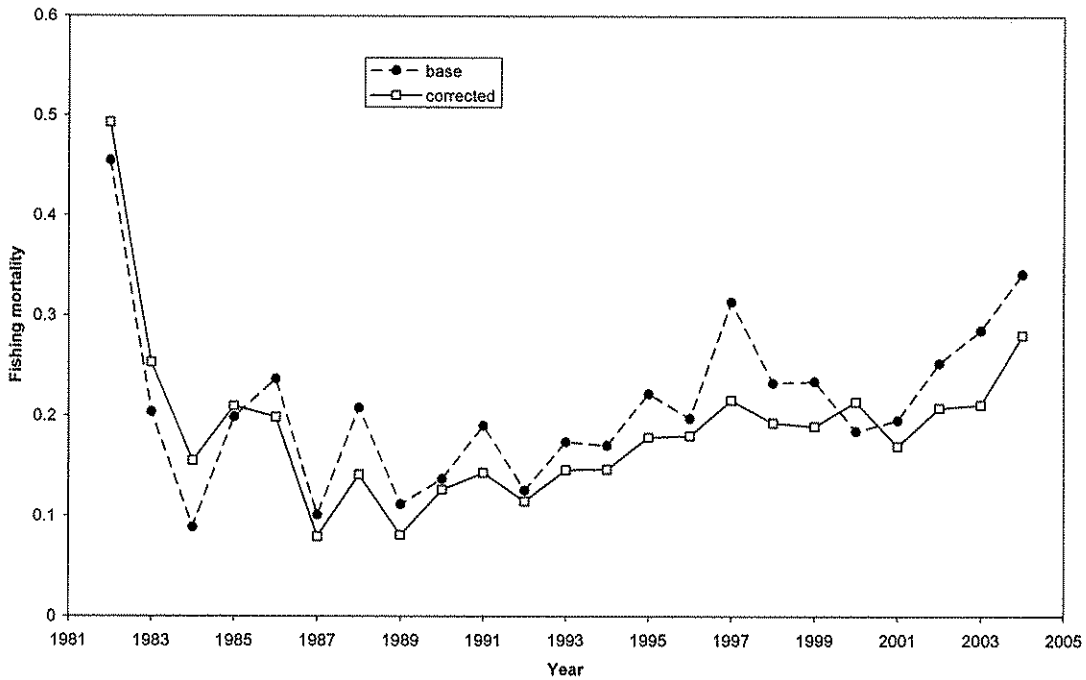


Figure 5. Estimates of number weighted fishing mortalities for ages 8-11 from the base and corrected run of ADAPT analysis.

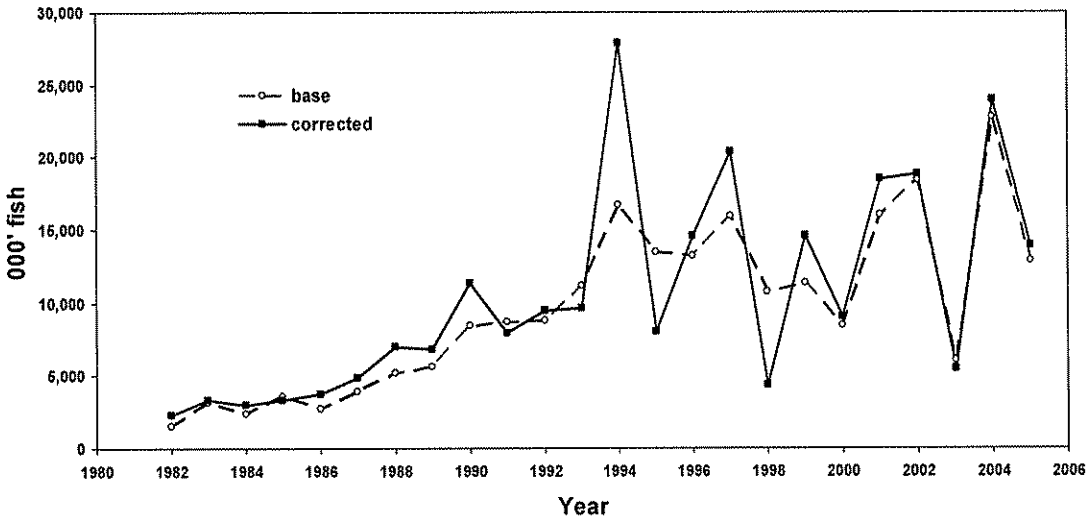


Figure 6. Comparison of recruitments estimated from the base and corrected run of ADAPT analysis.

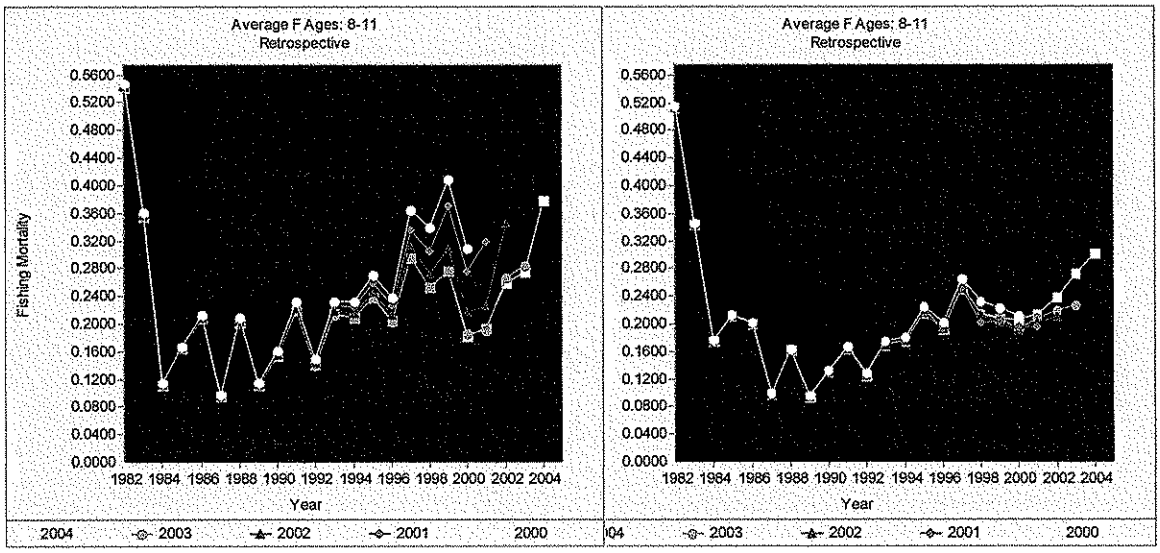


Figure 7. Comparison of retrospective analysis of the average fishing mortality for age 8-11 from the base and corrected runs. The left panel is the base run and the right panel is the corrected run.

Chapter 16

Methods and marginal increment analyusis validation for age estimation of spot (*Leiostomus xanthurus*) using their otoliths in Chesapeake Bay, Virginia

Introduction

The spot fishery is an important marine fishery along Atlantic coast of United States, ranging from Florida to New York. In 2006, the commercial landing was almost 3.2 million fish and valued for more than $2.6 million, and the recreational harvest was 11.1 million fish (Grist et al. 2007). Therefore, a research-based management plan for spot was important for the fishery and economy in the region.

In its Fishery Management Plan for Spot, the Atlantic States Marine Fisheries Commission (ASMFC) listed ageing criteria and validation for spot as one of its high research priorities in the future (Grist 2007). This is partially because older spot than expected have been reported in the previous years, and these older ages need validation. It is commonly thought that the maximum age for spot is 4 year, although DeVries (1982) reported that the maximum age of spot was 5 years. Moreover, spot of Age 6 have been reported in Virginia waters since 2003, implying that age structure and population dynamics of spot in this region may be changing. Obviously, ageing criteria and validation for spot ages in the region will enhance our understanding and provide confidence that these changes are real.

Several previous studies reported ageing criteria and validation for spot. Sundararaj (1960) validated both scales and otoliths as ageing methods for spot in Louisiana waters and Pacheco (1962) conducted validation of scales for ageing spot in Chesapeake Bay, Virginia. However, these ageing criteria and validation results are inappropriate to age spot using their otoliths in Virginia waters due to geographic variations and hard-part differences (Campana 2001). For example, Sundararaj (1960) reported that annulus formation on spot scales occurred between February and March in Louisiana waters whereas Pacheco (1962) found that annulus formation on spot scales occurred between January and February in Chesapeake Bay. In an example for other species, Machias et al. (1998) and Machias et al. (2002) found that annulus formation on scales occurred 2 to 3 months earlier than on otoliths of red porgy, *Pagrus pagrus*, and common dentex, *Dentex dentex*, respectively, in laboratory environments. Although Piner (1999) validated the annulus formation on otolith of spot for fish of age 0 to 2 collected in Chesapeake Bay, his results did not validate ages in older spot. Subsequent to an exhaustive search of the

literature, we have found no previous studies that have validated annuli formation in otoliths for spot older than age 2 in Chesapeake Bay, Virginia.

We are addressing the issue of spot ageing validation through three activities: 1) developing protocols for processing otoliths, 2) developing protocols for ageing spot with otoliths, and 3) providing a marginal increment analysis to validate time of annulus formation in Virginia waters. The first two have been completed during the fall of 2007 and the third is in process and will be complete by mid-spring 2008 in time for the ASMFC meeting to evaluate ageing in spot. The two protocols have been developed as printed literature to be handed out at the ASMFC meeting and they based on our 10 years of experience preparing otoliths for age estimation and on our ageing experience from the past several years. As we cross-checked the information in the protocols, we realized the lack of validation of otolith ageing for spot in Chesapeake Bay and Virginia waters at older ages. Previously, we determined that annuli formed on otoliths from May-July over the first two years of life (Piner 1999). Therefore, we initiated a project to provide a validation of older spot based on marginal-increment analysis using the data collected from the previous years. Our results will be presented to the spring meeting of ASMFC, and in our 2008 annual report to Virginia Marine Resources Commission in fall of 2008.

Preparation of otolith transverse cross-sections from spot for age estimation

Equipment

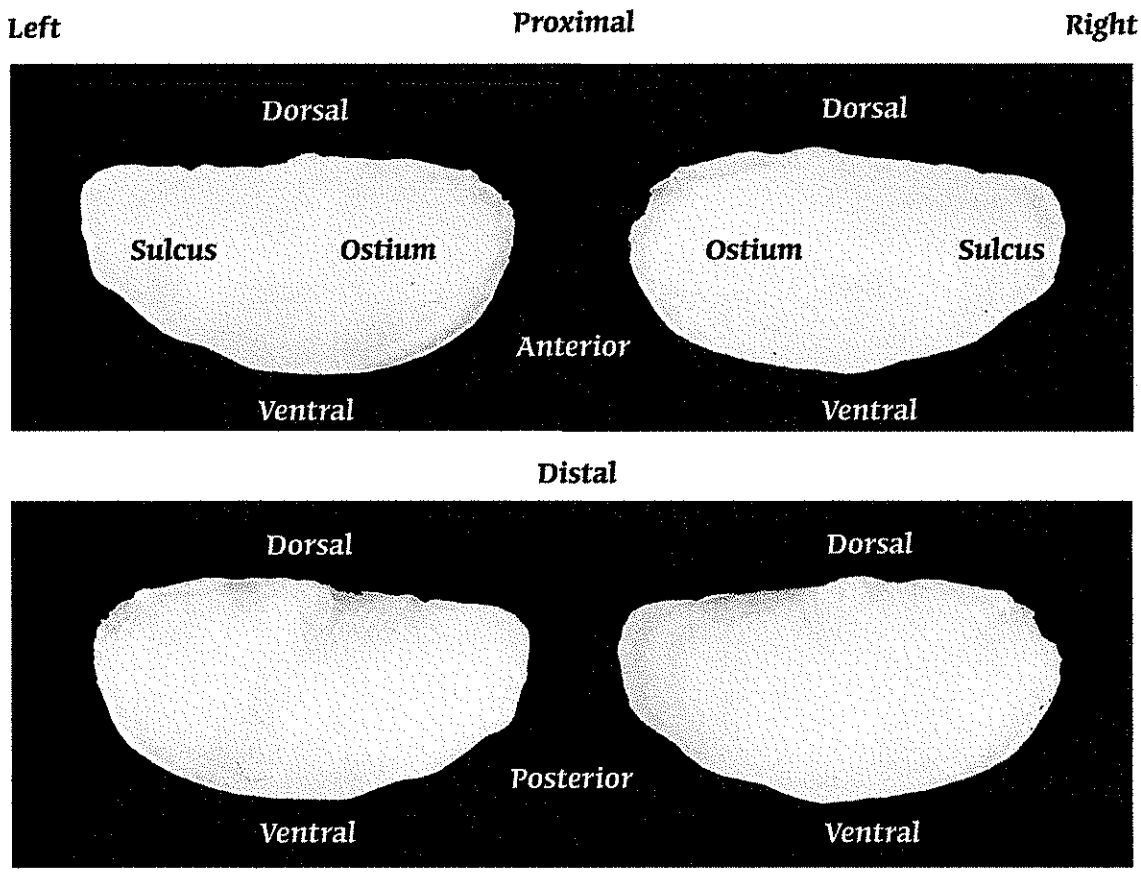
• Leica MZ 9_5 Stereo-microscope with transmitted light source	
• Buehler Isomet, low-speed saw	Model number 11-1280-160
• Ceramic top hot plate	VWR model number 115V, 1000W, 7 x 7"

Supplies

• Micro slide [1inch x 3 inches x 1.2mm]	1 per otolith plus extras
• Micro slide storage box	1 per 100 otoliths
• Aluminum slide tray	1 per 20 otoliths
• Glass eye dropper and dropping bottle	1
• Stender dish with lid for Crystalbond	1
• Dissecting forceps	2
• Diamond etcher	2
• Crystal bond 509-S	1 stick
• Flo-Texx liquid cover slip	1 quart

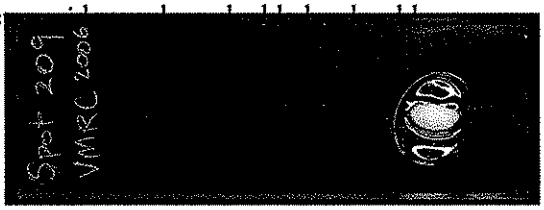
Structure of whole sagittal otoliths from spot

Sciaenids are characterized, among other things, by having extremely large sagittae relative to body size and a unique tadpole shaped sulcus with a massively enlarged ostium. The sulcus can be divided into two regions, the anterior region is called the ostium and the posterior region is called the cauda which typically curves downward in the spot otolith.



1. Mounting the spot otoliths for sectioning

Mount the otolith, distal side down, onto a micro slide using Crystal-bond. Make sure that the otolith lies parallel to the long edge of the slide. Allow the crystal-bond to cool. Mark the otolith with a fine tipped sharpie to designate the path of the saw blade. The mark should set up the cut so that the position of the nucleus is found in the transverse section to be removed from the otolith. The line is jus



Spot otolith mounted on slide

Spot otolith marked with cut line

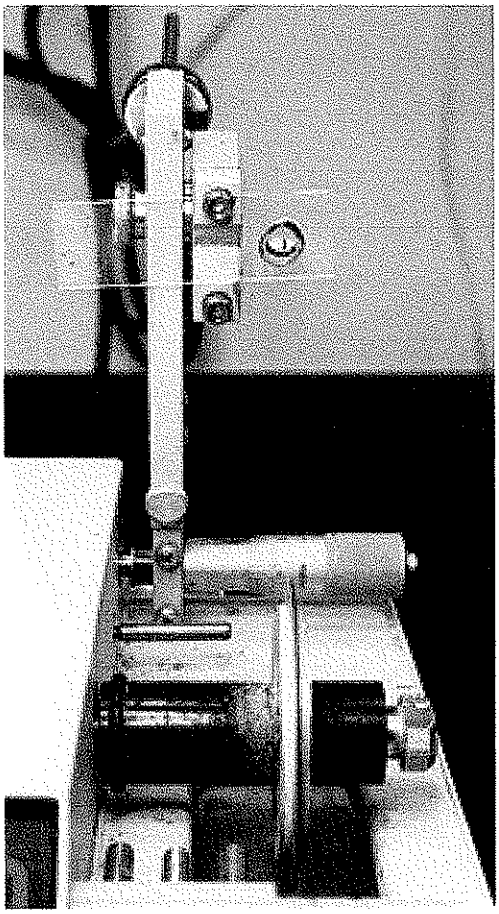
2. Sectioning the spot otoliths

Mount the slide in the Isomet saw arm brackets. Be sure that the cut line marked on the otolith runs parallel to the path of the saw blades.

Before cutting the otolith, make sure that the Isomet saw is set up with the recommended spacer, blades and weights.

Recommended set-up for the saw

One or two 25g weights (optional)
Two 2 ½ inch flanges
One 2 ½ inch 0.4 mm spacer
Two 3 inch diamond wheel blades (75 grit)



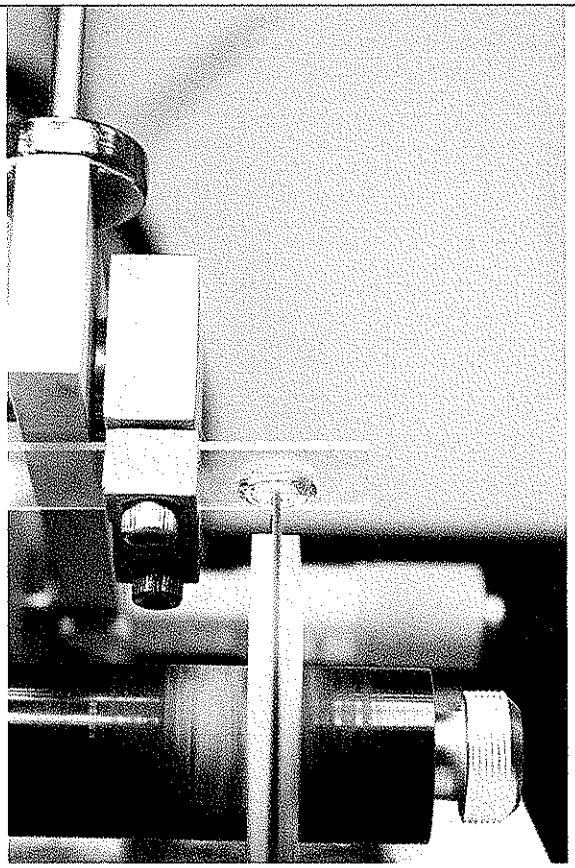
Spot otolith mounted on Isomet saw

When all of the Isomet saw specifications have been met, it is safe to start sectioning the otolith. Before positioning the otolith on the blades, start the Isomet saw at a speed of 3 or 4. Once the blades have established a groove in the otolith, bring the speed up to 7 or 8. The saw should now be cutting the otolith. This process should take about 2-3 minutes.

Weights and speed can be adjusted when working with more fragile or robust otoliths.

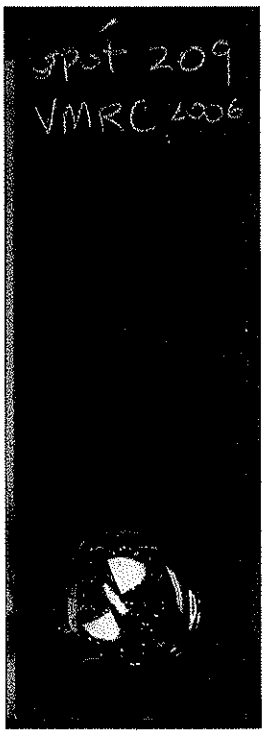
When the saw blades have cut through the entire otolith, but not through the Crystal bond, carefully lift the arm from the saw blades and turn off the saw.

The otolith should still contain the section that was just cut. To remove it, warm the crystal bond on a hot plate and gently remove the posterior and anterior sections from the slide first. This will free-up the cross section for easy removal from the Crystal Bond. Save the anterior and posterior pieces of otolith for later use.



Spot otolith on Isomet saw blade

Spot otolith warming on hot plate



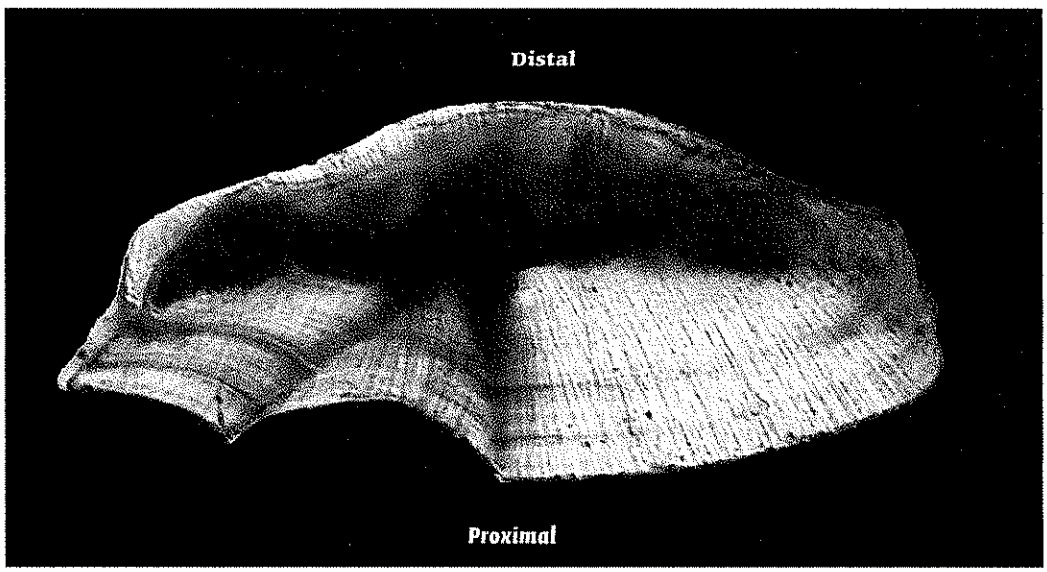
Removing the spot otolith section

3. Mounting the spot otolith section

Before permanently mounting the sections, look at them under a microscope and make sure that the best surface of the section is facing upwards and that there is no crystal bond causing it to sit unevenly on the slide. If crystal bond is apparent, warm the section to melt it away from the underside of the section.

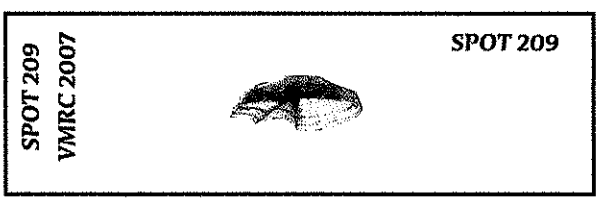
When the sections are dry, protect them with Flo-Texx, a liquid cover slip. Use an eyedropper to put a small amount of Flo-Texx over the section and spread it in a circular motion. Avoid creating bubbles as these will obscure the surface and interfere with reading the otoliths when estimating age. Eliminate bubbles while the Flo-Texx is still wet by popping them or moving them away from the otolith using tweezers. Allow the Flo-Texx to air dry on the otoliths.

Final results should look like the example below.



4. Storing the completed otolith sections

Use a diamond etcher to carve the species ID (SPOT), Age and Growth ID number, e.g., 209, VMRC, and the year, e.g., 2007, into the slide- parallel to its left, short side. Using a black Sharpie write the species ID and A&G ID in the upper right corner on the long side of the slide, on the opposite end of the etching. Store the slides in a labeled micro-slide box with the black Sharpie label along the top edge for easy identification.



Ageing spot useing otolith transverse cross-sections

Equipment & Supplies

- Leica MZ 9_5 Stereo-microscope with transmitted light source
- Mounted spot otolith transverse cross-sections on labeled microslides

General Spot Aging Procedure:

All fish are to be aged in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. Two readers must age each otolith independently. When the readers' ages agree, that age is to be assigned to the fish. When the two readers disagree, both readers must sit down together and re-age the fish, again without any knowledge of previously estimated ages or lengths, and assign a final age to the fish. When the readers are unable to agree on a final age, the fish must be excluded from further analysis.

Specific Spot Aging Procedure

Aging Spot otoliths involves two steps. First, the otolith transverse cross-section must be read: the number of annuli must be counted. Next, the age of the fish must be determined.

Step 1: Reading the Otolith

1) Remove a labeled slide with mounted Spot otolith transverse cross-section from the slide box labeled "Spot Otoliths (Year) VMRC."

2) Place the slide on the microscope stage and turn on the transmitted light source. Adjust the dark-field polarization (either from the stage or the lens, depending on the microscope) until a dark blue background appears behind the otolith section.

3) Adjust coarse and fine focus until the entire otolith is in clear view. A well-sectioned otolith will provide a clear view of the core and the succulus, a strong "V" shape extending from the core along which annual rings are plainly visible (Figure 1).

4) If the section lacks a clear "V" shape for the succulus, the cut did not go through the core and the otolith should be resectioned.

5) When a good section has been obtained, the first step is to count outward from the core each annulus visible on the section. Although an otolith annulus is actually the combination of an opaque and translucent band, when ageing otoliths only opaque lines are enumerated, and are referred to as annuli (Figure 1). Translucent areas of the section, then, represent growth between annuli. The first annulus on sectioned otoliths is typically quite distant from the core. Subsequent annuli appear regularly spaced along the dorsal and ventral arms of the sulcal groove, extending out towards the proximal (inner-face) edge of the otolith (Figure 1).

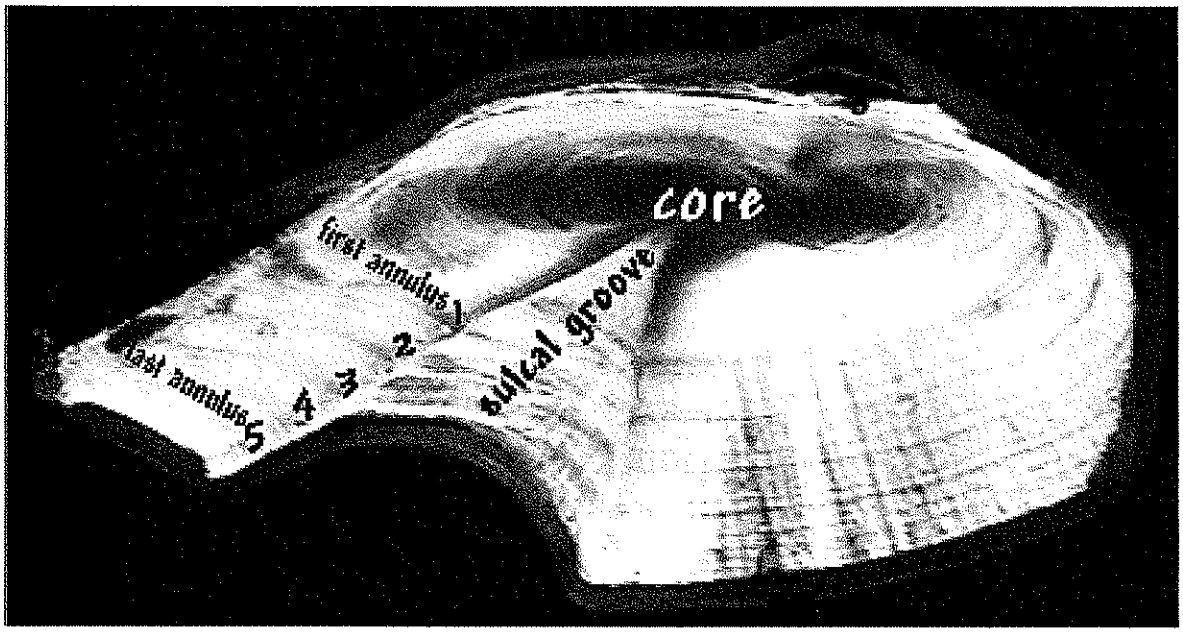


Figure 1
The core and sulcal groove are indicated by name in their respective locations. Numbers represent annuli, with one representing the first annulus on the otolith, and five representing the last annulus.

Step 2: Assigning an age to the fish

Once the number of annuli on the otolith has been identified, two scenarios shape the age determined for the fish:

1) There is no growth beyond the last annulus:
   The age of the fish becomes an even "X," where X is the number of annuli counted on the section in Step 1. This typically happens when a fish has been collected during the period when its annulus would be laid down. For Spot, annuli are laid down from May 1 to July 31 (Piner, 1999) (*Figure 2*).

2) There is growth beyond the last annulus:
   The growth is indicated by a "+" after the number of annuli, that is "X+."

   2.1) If the sacrifice date for the fish is between January 1 and the end of the last month in which Spot annuli are laid down (July 31), the age of the fish is represented as "X+ (X+1)." For example, a fish with 5 annuli visible on its otolith transverse cross-section would be assigned the age "5+ 6," indicating that it is 6 years old in its sacrifice year.

   2.2) If the sacrifice date for the fish falls after July 31 and before January 1, the fish has laid down its annulus for the year and has experienced growth since that time. The age of the fish is represented as "X+ (X)." A fish with 5 annuli visible on its otolith transverse cross-section would be assigned the age "5+ 5," indicating it is 5 years old in its sacrifice year.

Spot

Leiostomus xanthurus

Year	Year Zero									Year One													Year Two											
Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Birth date (Assigned) [1]	1/1												1/1												1/1											
Birth date (Actual) [2]													1/1												1/1											
Spawning period																																				
Annulus formation																																				
Number of Rings										0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
Biological age										0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
Year grouping										0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2

1 Assigned to all fishes born in the Northern Hemisphere.
2 Actual is average biological birth date for the species.

Figure 2

A graphical representation of the ageing data collected for Spot. Months are represented by their first letter within the gray axis. Months of spawning for spot are indicated in red (Warlen & Chester, 1985, Flores-Coto & Warlen, 1993; Powell & Gordy, 1980). Months of annulus formation are indicated in blue (Piner, 1999).

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