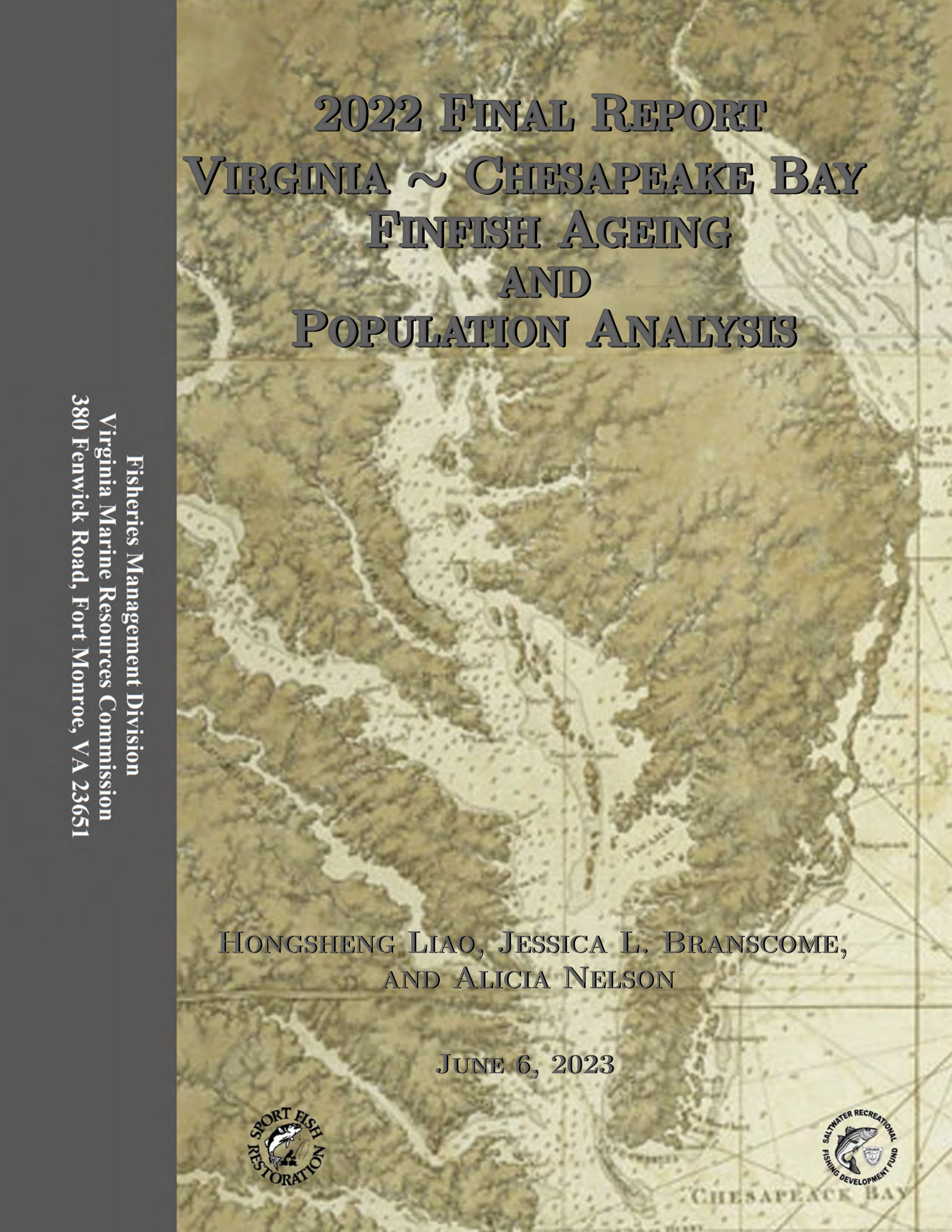
2022 Final Report
Virginia ~ Chesapeake Bay
Finfish Ageing
and
Population Analysis

Hongsheng Liao, Jessica L. Branscome,
and Alicia Nelson

Fisheries Management Division
Virginia Marine Resources Commission
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TABLE OF CONTENTS

EXECUTIVE SUMMARY xvi

ACKNOWLEDGMENTS xx

1 ATLANTIC CROAKER *Micropogonias undulatus* 1
- 1.1 INTRODUCTION . . . 2
- 1.2 METHODS . . . 2
  - 1.2.1 Sample Size for Ageing . . . 2
  - 1.2.2 Handling of Collections . . . 2
  - 1.2.3 Preparation . . . 2
  - 1.2.4 Readings . . . 3
  - 1.2.5 Comparison Tests . . . 4
- 1.3 RESULTS . . . 4
  - 1.3.1 Sample Size . . . 4
  - 1.3.2 Year Class . . . 4
  - 1.3.3 Age-length Key (ALK) . . . 5
  - 1.3.4 Reading Precision . . . 5

2 BLACK DRUM *Pogonias cromis* 10
- 2.1 INTRODUCTION . . . 11
- 2.2 METHODS . . . 11
  - 2.2.1 Handling of Collections . . . 11
  - 2.2.2 Preparation . . . 11
  - 2.2.3 Readings . . . 11
  - 2.2.4 Comparison Tests . . . 12
- 2.3 RESULTS . . . 13
  - 2.3.1 Year Class . . . 13
  - 2.3.2 Age-length Key (ALK) . . . 13
  - 2.3.3 Reading Precision . . . 13

3 BLUEFISH *Pomatomus saltatrix* 16
- 3.1 INTRODUCTION . . . 17
- 3.2 METHODS . . . 17
  - 3.2.1 Sample Size for Ageing . . . 17
  - 3.2.2 Handling of Collections . . . 17
  - 3.2.3 Preparation . . . 17
  - 3.2.4 Readings . . . 18
  - 3.2.5 Comparison Tests . . . 19
- 3.3 RESULTS . . . 20
  - 3.3.1 Sample Size . . . 20
  - 3.3.2 Year Class . . . 20
  - 3.3.3 Age-length Key (ALK) . . . 20
  - 3.3.4 Reading Precision . . . 20

4 COBIA *Rachycentron canadum* 29
4.1 INTRODUCTION . . . 30
4.2 METHODS . . . 30
4.2.1 Handling of Collections . . . 30
4.2.2 Preparation . . . 30
4.2.3 Readings . . . 30
4.2.4 Comparison Tests . . . 31
4.3 RESULTS . . . 32
4.3.1 Year Class . . . 32
4.3.2 Age-length Key (ALK) . . . 32
4.3.3 Reading Precision . . . 32

5 RED DRUM *Sciaenops ocellatus* 35
5.1 INTRODUCTION . . . 36
5.2 METHODS . . . 36
5.2.1 Handling of Collections . . . 36
5.2.2 Preparation . . . 36
5.2.3 Readings . . . 36
5.2.4 Comparison Tests . . . 37
5.3 RESULTS . . . 38
5.3.1 Year Class . . . 38
5.3.2 Age-length Key (ALK) . . . 38
5.3.3 Reading Precision . . . 38

6 SHEEPSHEAD *Archosargus probatocephalus* 42
6.1 INTRODUCTION . . . 43
6.2 METHODS . . . 43
6.2.1 Handling of Collections . . . 43
6.2.2 Preparation . . . 43
6.2.3 Readings . . . 43
6.2.4 Comparison Tests . . . 44
6.3 RESULTS . . . 45
6.3.1 Year Class . . . 45
6.3.2 Age-length Key (ALK) . . . 45
6.3.3 Reading Precision . . . 45

7 ATLANTIC SPADEFISH *Chaetodipterus faber* 48
7.1 INTRODUCTION . . . 49
7.2 METHODS . . . 49
7.2.1 Sample Size for Ageing . . . 49
7.2.2 Handling of Collections . . . 49
7.2.3 Preparation . . . 49
7.2.4 Readings . . . 50
7.2.5 Comparison Tests . . . 51
7.3 RESULTS . . . 51
7.3.1 Sample Size . . . 51
7.3.2 Year Class . . . 51
7.3.3 Age-length Key (ALK) . . . 51

7.3.4 Reading Precision . . . 51

8 SPANISH MACKEREL *Scomberomorous maculatus* 57
8.1 INTRODUCTION . . . 58
8.2 METHODS . . . 58
8.2.1 Sample Size for Ageing . . . 58
8.2.2 Handling of Collections . . . 58
8.2.3 Preparation . . . 58
8.2.4 Readings . . . 59
8.2.5 Comparison Tests . . . 60
8.3 RESULTS . . . 60
8.3.1 Sample Size . . . 60
8.3.2 Year Class . . . 60
8.3.3 Age-length Key (ALK) . . . 60
8.3.4 Reading Precision . . . 61

9 SPOT *Leiostomus xanthurus* 66
9.1 INTRODUCTION . . . 67
9.2 METHODS . . . 67
9.2.1 Sample Size for Ageing . . . 67
9.2.2 Handling of Collections . . . 67
9.2.3 Preparation . . . 67
9.2.4 Readings . . . 68
9.2.5 Comparison Tests . . . 69
9.3 RESULTS . . . 69
9.3.1 Sample Size . . . 69
9.3.2 Year Class . . . 69
9.3.3 Age-length Key (ALK) . . . 69
9.3.4 Reading Precision . . . 69

10 SPOTTED SEATROUT *Cynoscion nebulosus* 75
10.1 INTRODUCTION . . . 76
10.2 METHODS . . . 76
10.2.1 Sample Size for Ageing . . . 76
10.2.2 Handling of Collections . . . 76
10.2.3 Preparation . . . 76
10.2.4 Readings . . . 77
10.2.5 Comparison Tests . . . 78
10.3 RESULTS . . . 78
10.3.1 Sample Size . . . 78
10.3.2 Year Class . . . 78
10.3.3 Age-length Key (ALK) . . . 78
10.3.4 Reading Precision . . . 79

11 STRIPED BASS *Morone saxatilis* 84
11.1 INTRODUCTION . . . 85
11.2 METHODS . . . 85
11.2.1 Sample Size for Ageing . . . 85

11.2.2 Handling of Collection . . . 85
11.2.3 Preparation . . . 85
11.2.3.1 *Otoliths* . . . 85
11.2.3.2 *Scales* . . . 86
11.2.4 Readings . . . 86
11.2.4.1 *Otoliths* . . . 87
11.2.4.2 *Scales* . . . 88
11.2.5 Comparison Tests . . . 88
11.3 RESULTS . . . 89
11.3.1 Sample Size . . . 89
11.3.1.1 *Chesapeake Bay* . . . 89
11.3.1.2 *Atlantic Ocean* . . . 89
11.3.2 Year Class . . . 89
11.3.2.1 *Chesapeake Bay* . . . 90
11.3.2.2 *Atlantic Ocean* . . . 90
11.3.3 Age-Length-Key (ALK) . . . 90
11.3.4 Reading Precision . . . 90
11.3.4.1 *Otoliths* . . . 90
11.3.4.2 *Scales* . . . 91
11.3.5 Comparison of Scale and Otolith Ages . . . 91
11.4 RECOMMENDATIONS . . . 92

12 SUMMER FLOUNDER *Paralichthys dentatus* 101
12.1 INTRODUCTION . . . 102
12.2 METHODS . . . 102
12.2.1 Sample Size for Ageing . . . 102
12.2.2 Handling of Collection . . . 102
12.2.3 Preparation . . . 102
12.2.3.1 *Otoliths* . . . 102
12.2.3.2 *Scales* . . . 103
12.2.4 Readings . . . 103
12.2.4.1 *Otoliths* . . . 104
12.2.4.2 *Scales* . . . 105
12.2.5 Comparison Tests . . . 106
12.3 RESULTS . . . 106
12.3.1 Sample Size . . . 106
12.3.1.1 *Chesapeake Bay* . . . 106
12.3.1.2 *Atlantic Ocean* . . . 106
12.3.2 Year class . . . 106
12.3.2.1 *Chesapeake Bay* . . . 106
12.3.2.2 *Atlantic Ocean* . . . 107
12.3.3 Age-Length-Key (ALK) . . . 107
12.3.4 Reading Precision . . . 107
12.3.4.1 *Otoliths* . . . 107
12.3.4.2 *Scales* . . . 108
12.3.5 Comparison of Scale and Otolith Ages . . . 108
12.4 RECOMMENDATIONS . . . 108

13 TAUTOG *Tautoga onitis* **118**
13.1 INTRODUCTION . . . 119
13.2 METHODS . . . 119
13.2.1 Sample Size for Ageing . . . 119
13.2.2 Handling of Collection . . . 119
13.2.3 Hardpart Preparation . . . 119
13.2.3.1 *Otoliths* . . . 119
13.2.3.2 *Opercula* . . . 120
13.2.3.3 *Spines* . . . 120
13.2.4 Readings . . . 120
13.2.4.1 *Otoliths* . . . 121
13.2.4.2 *Opercula* . . . 121
13.2.4.3 *Spines* . . . 121
13.2.5 Comparison Tests . . . 122
13.3 RESULTS . . . 122
13.3.1 Sample Size . . . 122
13.3.1.1 *Chesapeake Bay* . . . 122
13.3.1.2 *Atlantic Ocean* . . . 123
13.3.2 Year Class . . . 123
13.3.2.1 *Chesapeake Bay* . . . 123
13.3.2.2 *Atlantic Ocean* . . . 123
13.3.3 Age-Length-Key (ALK) . . . 123
13.3.4 Reading Precision . . . 124
13.3.4.1 *Otoliths* . . . 124
13.3.4.2 *Opercula* . . . 124
13.3.4.3 *Spines* . . . 124
13.3.5 Comparisons . . . 125
13.3.5.1 *Operculum vs otolith ages* . . . 125
13.3.5.2 *Spine vs otolith ages* . . . 125
13.4 RECOMMENDATIONS . . . 126

14 WEAKFISH *Cynoscion regalis* **134**
14.1 INTRODUCTION . . . 135
14.2 METHODS . . . 135
14.2.1 Sample Size for Ageing . . . 135
14.2.2 Handling of Collections . . . 135
14.2.3 Preparation . . . 135
14.2.4 Readings . . . 136
14.2.5 Comparison Tests . . . 137
14.3 RESULTS . . . 137
14.3.1 Sample Size . . . 137
14.3.2 Year Class . . . 137
14.3.3 Age-length Key (ALK) . . . 137
14.3.4 Reading Precision . . . 138

REFERENCES **143**

APPENDIX **145**

LIST OF TABLES

Table 1 The minimum and maximum ages, number of fish and their hardparts collected, number of fish aged, and age readings (by both readers) for the 14 finfish species in 2022. Besides otoliths, the hardparts and age readings include scales for Striped Bass and Summer Flounder, and both opercula and spines for Tautog. The otolith-ages are reported for all the species. When otolith-ages are not available, scale-ages are reported for Striped Bass and Summer Flounder whereas operculum-ages are reported for Tautog. However, when neither otolith- nor operculum-ages are avaialbe for Tautog, spine-ages are reported. xvii

Table 1.1 Number of Atlantic Croaker collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 6

Table 1.2 CV for each age estimated based on ageing the total of 374 Croaker in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Croaker collected from 2016 to 2020. 7

Table 1.3 The number of Atlantic Croaker assigned to each total length-at-age category for 226 fish sampled for otolith age determination in Virginia during 2022. 8

Table 1.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Croaker sampled for age determination in Virginia during 2022. 9

Table 2.1 The number of Black Drum assigned to each total length (inch)-at-age category for 107 fish sampled for otolith age determination in Virginia during 2022. 14

Table 2.2 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Black Drum sampled for age determination in Virginia during 2022. 15

Table 3.1 Number of Bluefish collected and aged in each 1-cm length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 22

Table 3.2 CV for each age estimated based on ageing the total of 421 Bluefish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Bluefish collected from 2016 to 2020. 24

Table 3.3 The number of Bluefish assigned to each total length (cm)-at-age category for 297 fish sampled for otolith age determination in Virginia during 2022. 25

Table 3.4 Age-Length key, as proportion-at-age in each 1-cm length interval, based on otolith ages for Bluefish sampled for age determination in Virginia during 2022. 27

Table 4.1 The number of Cobia assigned to each total length (inch)-at-age category for 327 fish sampled for otolith age determination in Virginia during 2022. 33

Table 4.2 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Cobia sampled for age determination in Virginia during 2022. . . . 34

Table 5.1 The number of Red Drum assigned to each total length (inch)-at-age category for 93 fish sampled for otolith age determination in Virginia during 2022. 40
Table 5.2 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Red Drum sampled for age determination in Virginia during 2022. 41

Table 6.1 The number of sheepshead assigned to each total length (inch)-at-age category for 469 fish sampled for otolith age determination in Virginia during 2022. 46
Table 6.2 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Sheepshead sampled for age determination in Virginia during 2022. 47

Table 7.1 Number of Atlantic Spadefish collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 53
Table 7.2 CV for each age estimated based on ageing the total of 357 Spadefish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spadefish collected from 2016 to 2020. 54
Table 7.3 The number of Atlantic Spadefish assigned to each total length-at-age category for 249 fish sampled for otolith age determination in Virginia during 2022. 55
Table 7.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Spadefish sampled for age determination in Virginia during 2022. 56

Table 8.1 Number of Spanish Mackerel collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 62
Table 8.2 CV for each age estimated based on ageing the total of 228 Spanish Mackerel in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spanish Mackerel collected from 2016 to 2020. 63
Table 8.3 The number of Spanish Mackerel assigned to each total length-at-age category for 210 fish sampled for otolith age determination in Virginia during 2022. 64
Table 8.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spanish Mackerel sampled for age determination in Virginia during 2022. 65

Table 9.1 Number of Spot collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 71
Table 9.2 CV for each age estimated based on ageing the total of 170 Spot in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spot collected from 2016 to 2020. 72
Table 9.3 The number of Spot assigned to each total length-at-age category for 172 fish sampled for otolith age determination in Virginia during 2022. 73
Table 9.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spot sampled for age determination in Virginia during 2022. . . . 74

Table 10.1 Number of Spotted Seatrout collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 80
Table 10.2 CV for each age estimated based on ageing the total of 299 Spotted Seatrout in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spotted Seatrout collected from 2016 to 2020. 81
Table 10.3 The number of Spotted Seatrout assigned to each total length-at-age category for 283 fish sampled for otolith age determination in Virginia during 2022. 82
Table 10.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spotted Seatrout sampled for age determination in Virginia during 2022. 83

Table 11.1 Number of bay Striped Bass collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 93
Table 11.2 CV for each age estimated based on ageing the total of 530 bay Striped Bass in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Striped Bass collected from 2016 to 2020. 94
Table 11.3 Number of ocean Striped Bass collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 95
Table 11.4 CV for each age estimated based on ageing the total of 501 ocean Striped Bass in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Striped Bass collected from 2016 to 2020. 96
Table 11.5 The number of Striped Bass assigned to each total length-at-age category for 527 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2022. 97
Table 11.6 The number of Striped Bass assigned to each total length-at-age category for 293 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2022. 98
Table 11.7 Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Chesapeake Bay, Virginia during 2022. 99
Table 11.8 Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Virginia waters of the Atlantic Ocean during 2022. 100

Table 12.1 Number of bay Summer Flounder collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 110
Table 12.2 CV for each age estimated based on ageing the total of 381 bay Summer Flounder in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Summer Flounder collected from 2016 to 2020. 111

Table 12.3 Number of ocean Summer Flounder collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 112
Table 12.4 CV for each age estimated based on ageing the total of 478 ocean Summer Flounder in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Summer Flounder collected from 2016 to 2020. 113
Table 12.5 The number of Summer Flounder assigned to each total length-at-age category for 375 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2022. 114
Table 12.6 The number of Summer Flounder assigned to each total length-at-age category for 470 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2022. 115
Table 12.7 Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Chesapeake Bay, Virginia during 2022. 116
Table 12.8 Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Virginia waters of the Atlantic Ocean during 2022. 117

Table 13.1 Number of bay Tautog collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 127
Table 13.2 CV for each age estimated based on ageing the total of 452 bay Tautog in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Tautog collected from 2016 to 2020. 128
Table 13.3 Number of ocean Tautog collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 129
Table 13.4 CV for each age estimated based on ageing the total of 454 ocean Tautog in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Tautog collected from 2016 to 2020. 130
Table 13.5 The number of Tautog assigned to each total length-at-age category for 174 fish sampled for both otolith and operculum age determination in Chesapeake Bay, Virginia during 2022. 131
Table 13.6 The number of Tautog assigned to each total length-at-age category for 7 fish sampled for both otolith and operculum age determination in Virginia waters of Atlantic ocean during 2022. 132
Table 13.7 Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and operculum ages for Tautog sampled in Chesapeake Bay, Virginia during 2022. 133

Table 14.1 Number of Weakfish collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. 139

Table 14.2 CV for each age estimated based on ageing the total of 326 Weakfish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Weakfish collected from 2016 to 2020. 140
Table 14.3 The number of Weakfish assigned to each total length-at-age category for 235 fish sampled for otolith age determination in Virginia during 2022. 141
Table 14.4 Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Weakfish sampled for age determination in Virginia during 2022. . 142
Table A1 Sample sizes of the length data collected from commercial fisheries by fleet and year. A-9
Table A2 Sample sizes of the age-length data collected from coast-wide, by region, state, and year. A-10

LIST OF FIGURES

Figure 1.1 Otolith thin-sections of a 8 year-old Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section 4
Figure 1.2 Year-class frequency distribution for Atlantic Croaker collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling. . . 4
Figure 1.3 Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 5

Figure 2.1 Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum. 12
Figure 2.2 Year-class frequency distribution for Black Drum collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 13
Figure 2.3 Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 13

Figure 3.1 Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section 19
Figure 3.2 Year-class frequency distribution for Bluefish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 20
Figure 3.3 Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 20

Figure 4.1 Otolith thin-section of a 4 year-old Cobia. 31
Figure 4.2 Year-class frequency distribution for Cobia collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 32
Figure 4.3 Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 32

Figure 5.1 Otolith thin-section of a 3 year-old Red Drum with the last annulus on the edge of the thin-section 37
Figure 5.2 Year-class frequency distribution for Red Drum collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 38
Figure 5.3 Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 38

Figure 6.1 Otolith thin-section of a 5 year-old Sheepshead 44

Figure 6.2 Year-class frequency distribution for Sheepshead collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 45
Figure 6.3 Between-reader comparison of otolith age estimates for Sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 45

Figure 7.1 Otolith thin-section of a 2 year-old Spadefish 51
Figure 7.2 Year-class frequency distribution for Spadefish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 52
Figure 7.3 Between-reader comparison of otolith age estimates for Spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 52

Figure 8.1 Otolith thin-section of a 3 year-old Spanish Mackerel with the last annulus on the edge of the thin-section 59
Figure 8.2 Year-class frequency distribution for Spanish Mackerel collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 60
Figure 8.3 Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 61

Figure 9.1 Otolith thin-section of a 2 year-old Spot 68
Figure 9.2 Year-class frequency distribution for Spot collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling. 69
Figure 9.3 Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 70

Figure 10.1 Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section 77
Figure 10.2 Year-class frequency distribution for Spotted Seatrout collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 78
Figure 10.3 Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 79

Figure 11.1 Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section 87
Figure 11.2 Scale impression of a 3 year-old Striped Bass. 88
Figure 11.3 Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling. 90

Figure 11.4 Year-class frequency distribution for Striped Bass collected in Virginia waters of the Atlantic Ocean for ageing in 2022. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling. 90
Figure 11.5 Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 91
Figure 11.6 Between-reader comparison of scale age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 91
Figure 11.7 Comparison of scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 92
Figure 11.8 Age-bias plot for Striped Bass scale and otolith age estimates in 2022. The number above the upper CI bar is number of fish. 92

Figure 12.1 Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section 104
Figure 12.2 Scale impression of a 1 year-old Summer Flounder 105
Figure 12.3 Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 107
Figure 12.4 Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2022. Distribution is broken down by. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 107
Figure 12.5 Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 108
Figure 12.6 Between-reader comparison of scale age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 108
Figure 12.7 Comparison of scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish 109
Figure 12.8 Age-bias plot for Summer Flounder scale and otolith age estimates in 2022. The number above the upper CI bar is number of fish. 109

Figure 13.1 Otolith thin-section of 6 year-old Tautog 121
Figure 13.2 Operculum of a 7 year-old Tautog 122
Figure 13.3 Spine of a 4 year-old Tautog 122
Figure 13.4 Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling. 123

Figure 13.5 Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 124
Figure 13.6 Between-reader comparison of operculum age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 124
Figure 13.7 Between-reader comparison of spine age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 125
Figure 13.8 Comparison of operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 125
Figure 13.9 Age-bias plot for Tautog operculum and otolith age estimates in 2022. The number above the upper CI bar is number of fish. 126
Figure 13.10 Comparison of spine and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 126
Figure 13.11 Age-bias plot for Tautog spine and otolith age estimates in 2022. The number above the upper CI bar is number of fish. 126

Figure 14.1 Otolith thin-section of 4 year-old Weakfish 136
Figure 14.2 Year-class frequency distribution for Weakfish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling. 137
Figure 14.3 Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish. 138
Figure A1 The relationship between fork and total length (mm) of Black Drum. A-11
Figure A2 Comparison in the total length of Black Drum between gears within each state. A-12
Figure A3 Tukey tests on the total length of Black Drum between gears within each state which has more than two gears. Two or more gears share the same letter are not significantly different. A-13
Figure A4 Comparison in the total length of Black Drum between states within each gear. A-14
Figure A5 Tukey tests on the total length of Black Drum between states within each gear which has more than two states. Two or more states share the same letter are not significantly different. A-15
Figure A6 NC Black Drum length distribution (1-inch interval) collected from NC commercial fisheries from 2008 to 2019. . . . . .A-16
Figure A7 Coastal wide Black Drum length distribution (1-inch interval) collected from both commercial and recreational fisheries from 2008 and 2019. . . . . .A-17
Figure A8 Age-length data before and after outlier removal by sex using boxplot function. "F" and "M" stand for female and male, respectively. One red circle represents one fish identified as an outlier. A-18
Figure A9 Kimura test on von Bertalanffy growth rates between coast wide and year-pooled female and male Black Drum. "F" and "M" stand for female and male, respectively. A data point is a mean length at age. A-19

Figure A10 Age-length data before and after outlier removal by region using boxplot function. Mid-Atlantic includes NE, MD, and VA whereas South Atlantic includes NC, SC, GA, and FL. One red circle represents one fish identified as an outlier. A-20
Figure A11 Kimura test on von Bertalanffy growth rates between coast wide and year-pooled Mid- and South Atlantic Black Drum. Mid-Atlantic includes NE, MD, and VA whereas South Atlantic includes NC, SC, GA, and FL. A data point is a mean length at age. A-21
Figure A12 Coastal wide age-length data before and after outlier removal by year using boxplot function. One red circle represents one fish identified as an outlier. . . . A-22
Figure A13 Coast-wide annual age distributions after outliers removed. "C", "FI", and "R" stand for the data collected from commercial fisheries, fishery independent survey, and recreational fisheries, respectively. A-23
Figure A14 NC annual age distributions from 2008 to 2019 converted from NC annual length distributions using coast-wide annual ALKs. A-24
Figure A15 Coast-wide annual age distributions from 2008 to 2019 converted from coast-wide annual length distributions using coast-wide annual ALKs. A-25
Figure A16 Coast-wide annual age distributions from 2008 to 2019 with removal of fish younger than Age 4. . . . . A-26
Figure A17 NC abundance indices. X-axis is year-class. A-27
Figure A18 NC trammel net CPUE index for Age 1 of Black Drum. A-28
Figure A19 Outliers were moved from the coast-wide year- and sex-combined age-length data collected between 1983 and 2021 from recreational, commercial fisheries, and fishery-independent surveys. A red circle represents one fish identified as an outlier. A-29
Figure A20 von Bertalanffy growth curve (blue line) with its parameters estimated using the region-, year-, and sex-pooled mean length-at-age data (red circles) collected between 1983 and 2020. The number in parenthesis is the sample size. The minimum age is 0 whereas the maximum age is 67. . . . . A-30
Figure A21 von Bertalanffy growth curve (blue line) with its parameters estimated using the region-, year-, and sex-pooled individual length-at-age data (red circles) collected between 1983 and 2020. The number in parenthesis is the sample size. The minimum age is 0 whereas the maximum age is 67. . . . . A-31

EXECUTIVE SUMMARY

This executive summary briefly summarizes what the Age and Growth Lab achieved in 2022 in terms of the objectives listed in the 2022 - 2023 proposal.

Objective 1: We propose to continue support of the Virginia Marine Resources Commission (VMRC) Age and Growth Laboratory, which is dedicated to providing Virginia fisheries management with reliable age estimates of marine fishes as an ongoing long-term activity. This includes yearly reports of catch-at-age of Virginia's important finfishes that are mandated by law, along with proper protocols to insure accuracy of the age estimates.

This objective is the major task the Age and Growth Lab is funded for, therefore, 14 chapters in the report are about the objective and each chapter is for one of 14 species the lab aged in 2022. We present the ageing results of 14 finfish species collected from commercial and recreational catches made in the Chesapeake Bay and Virginia waters of the Atlantic Ocean, U.S.A. in 2022. All fish were collected by the VMRC Biological Sampling Program in 2022 and aged in 2023 at the Age and Growth Laboratory of VMRC. We present age composition tables, graphs of year-class distributions, age-length keys, and measures of ageing precision for each species.

Four calcified structures (hard-parts) are used in age determination. More specifically, three calcified structures, otoliths, opercula, and pelvic spines (newly added in 2022), were used for determining fish ages of Tautog *Tautoga onitis* (n = 181). Two calcified structures, otoliths and scales, were used for determining fish ages of Striped Bass *Morone saxatilis* (n = 820) and Summer Flounder *Paralichthys dentatus* (n = 845). Comparing alternative hard-parts allowed us to assess their usefulness in determining fish age as well as the relative precision of each structure. Ages were determined from otoliths only for the following species: Atlantic Croaker *Micropogonias undulatus* (n = 226), Black Drum *Pogonias cromis* (n = 107), Bluefish *Pomatomus saltatrix* (n = 297), Cobia *Rachycentron canadum* (n = 327), Red Drum *Sciaenops ocellatus* (n = 93), Sheepshead *Archosargus probatocephalus* (n = 469), Atlantic Spadefish *Chaetodipterus faber* (n = 249), Spanish Mackerel *Scomberomorous maculates* (n = 210), Spot *Leiostomus xanthurus* (n = 172), Spotted Seatrout *Cynoscion nebulosus* (n = 283), and Weakfish *Cynoscion regalis* (n = 235). In total, we made 11,194 age readings from otoliths, scales, opercula, and spines collected during 2022. A summary of the age ranges for all species aged is presented in Table 1.

In 2022 we added Tautog spines in our ageing list, as a result, we aged not only their opercula and otoliths as before, we also aged their spines as well. This allowed us to compare the precision in ageing between these three hardparts for Tautog. In addition to finishing sectioning and ageing Tautog spines collected in 2022, we also started to section Tautog spines collected before 2022. Before the submision of this report, we have finished sectioning all backlogged Tautog spines (collected in 2019, 2020, and 2021).

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

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

Table 1: The minimum and maximum ages, number of fish and their hardparts collected, number of fish aged, and age readings (by both readers) for the 14 finfish species in 2022. Besides otoliths, the hardparts and age readings include scales for Striped Bass and Summer Flounder, and both opercula and spines for Tautog. The otolith-ages are reported for all the species. When otolith-ages are not available, scale-ages are reported for Striped Bass and Summer Flounder whereas operculum-ages are reported for Tautog. However, when neither otolith- nor operculum-ages are avaialbe for Tautog, spine-ages are reported.

Species	Number of fish collected	Number of hard-parts	Numnber of fish aged	Number of read-ings	Minimum age	Maximum age
Atlantic Croaker	282	282	226	452	0	8
Black Drum	107	107	107	214	0	49
Bluefish	377	376	297	594	0	8
Cobia	327	327	327	654	2	10
Red Drum	93	93	93	186	0	49
Sheepshead	469	469	469	938	1	40
Spadefish	251	249	249	498	0	8
Spanish Mackerel	295	295	210	420	0	7
Spot	197	197	172	344	0	4
Spotted Seatrout	549	549	283	566	0	5
Striped Bass	1,069	1,374	820	2,250	2	29
Summer Flounder	972	1,391	845	2,528	1	14
Tautog	181	541	181	1,080	2	24
Weakfish	247	245	235	470	1	5
Totals	5,416	6,495	4,514	11,194		

our efficiency and effectiveness on ageing those species. In 2022 we developed two Excel macros (VBA) to monitor the number of fish/carcasses and hardparts processed, respectively, in the lab on a weekly basis.

Objective 3: VMRC will develop routine stock assessments based on age-structured models (such as SVPA, ADAPT, Stock Synthesis, and AD Model Builder, among others where appropriate). Following several years of accumulation of aged-catch data, age-structured stock assessment models will be developed and periodically updated.

The purpose of this objective is to prepare VMRC to make contributions to stock assessment of any species along Atlantic coast when requested by Atlantic States Marine Fisheries Commission (ASMFC) and Southeast Data, Assessment and Review (SEDAR). ASMFC started to conduct the benchmark stock assessment for Black Drum in 2021, and continued it during 2022. In the Black Drum Benchmark Stock Assessment, Dr. Liao explored if there were sufficient age data to support any age-structured stock assessment model for Black Drum. Even though he found that there were not sufficient age data for any age-structured stock assessment model for Black Drum, he did find that the current age data could be used to track strong cohort progression through years and to monitor the stock abundance trend identified by the abundance indices used in the stock assessment. In 2022, Dr. Liao submitted his findings as a working paper to the Black Drum Benchmark Stock Assessment Committee (Please see the APPENDIX).

Objective 4: Develop VMRC Age and Growth Laboratory web pages at VMRC web site to publish protocols, other aids such as pictures of aged otoliths for all species, and other information to assist

other states and laboratories in the methods of ageing marine fishes.

Throughout the years we have continued to work on the design and content of a web page that promotes VMRC's efforts to properly manage Virginia's marine resources through our age and growth research. In addition to educating the public on the importance of ageing fishes, the web page has been of interest to fishermen for it provides fundamental information of the life history of Virginia's fishes. We posted VMRC 2021 Ageing Lab Final Report, providing the detailed information on what the ageing lab is about, what we do in the lab, and what contributions the ageing lab makes to the coast-wide marine fisheries management. We also posted Weakfish Otolith Removal Video, demonstrating how we remove Weakfish otoliths in the Ageing Lab.

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

In 2022, we developed and posted a new web-based application (VMRC Ageing Lab Image Share App). This app allows us to share large images (such as otolith thin-section image) made by the lab with other agencies while the images are too big to be emailed even using a zip-file. We updated age-length data in VMRC four web applications (Fish Age Estimator, Fish Growth Predictor, VMRC/CQFE Database App, and %MSP/%Female_SPR/%SPR Estimator). These apps help fishermen to understand the importance of knowledge on fish ages and growth, and allow fishermen and fisheries scientists to easily access and download the age and biological databases of 14 marine finfish species collected by VMRC at Chesapeake Bay and Virginia waters of Atlantic ocean from as early as 1998 to 2022 and aged by the lab. We revised the fish growth app (Fish Growth Predictor) so that the users can use not only the age-length data of the species collected by VMRC but also users' own fish age-length data to develop growth curves.

We continued to share VMRC Striped Bass otolith thin-section slides and their age data with Massachusetts Division of Marine Fisheries for its marginal increment analysis of Striped bass in Chesapeake Bay. In July of 2022 we provided Virginia Polytechnic Institute and State University (Virginia Tech) with the histological images of Blueline Tilefish *Caulolatilus microps* stored in the VMRC Ageing Lab database. Virginia Tech used those images to conduction a research on the histological reproductive phase determination of Blueline Tilefish. In April of 2023 We prepared the figures demonstrating the length-age relationships of Atlantic Croaker and Spot from 1998 to 2022 for the ASMFC Atlatnic Croaker and Spot Stock Assessment. We made a series of computer programs using Excel macro (VBA), Virtual Basic Script (VBS), and Task Scheduler to automatically update the ages in all the apps which use the age data produced by the Ageing and Growth Lab yearly, and automatically update the donation information in the donation app (Sportfish Donation Data) donated by Virginia recreational anglers monthly.

Objective 6: We will continue the publication of our results on accuracy and precision of ageing important marine finfish species, and their effects on stock assessments and fisheries management in scientific literature.

We continued to update the Ageing Lab Operation Protocol in 2022. Each time we revise an old processing method or add a new method, we update the protocol. In July of 2022, Dr. Liao, Lab Manager, participated the ASMFC Black Drum Benchmark Stock Assessment Workshop at Arlington, VA. In October of 2022, Dr. Liao attended the conference call of Black Drum TC/SAS on reviewing the final report of Black Drum Benchmark Stock Assessment. In March of 2023, Jessica Branscome, Chief Technician, participated the ASMFC Ageing Workshop on several species (Black Seabass, Bluefish, Cobia, Red Drum, Scup, Tautog, and Weakfish) at St. Petersburg, FL.

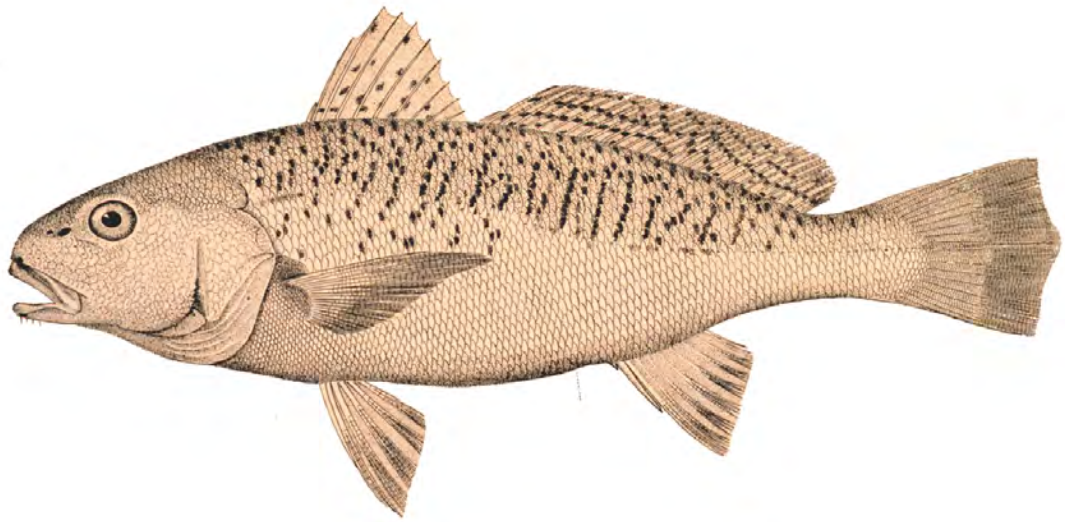
Besides above work the Age and Growth Lab did in 2022, to support environmental and wildlife agencies, and charities, we donated more than 2,730 pounds of dissected fish to the Salvation Army to feed the homeless, and Alton's Keep WildBird Rescue and Rehabilitation Center Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public.

ACKNOWLEDGMENTS

We thank Emily Davis, Marben Abutin, Kate Draa, and Kirsten Travis for their technical expertise in preparing otoliths, scales, opercula, and spines for age determination. They all put in long hours processing "tons" of fish in our lab. We would like also to thank the VMRC field technicians, Richard Hancock, Myra Thompson, and Chris Williams for their many efforts in this cooperative project. A special note of appreciation is extended to Ethan Simpson, VMRC Biological Sampling Program Supervisor, for his help in processing fish, collecting hardparts, and many other lab activities whenever we were short of hands in the lab.

Chapter 1

ATLANTIC CROAKER *Micropogonias undulatus*



1.1 INTRODUCTION

We aged a total of 226 Atlantic Croaker *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Croaker ages ranged from 0 to 8 years old with an average age of 2.2, a standard deviation of 1.2, and a standard error of 0.08. Nine age classes (0 to 8) were represented, comprising fish of the 2014 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2020 and 2021 with 48.2% and 27%, respectively.

1.2 METHODS

1.2.1 Sample Size for Ageing

We estimated sample size for ageing Croaker in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Croaker in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Croaker used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Croaker collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

1.2.2 Handling of Collections

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

1.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Barbieri et al. (1993) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

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

1.2.4 Readings

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

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

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

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

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 1.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section. However, due to discrepancy on identification of the first annulus of Atlantic Croaker among Atlantic states, ASMFC has decided not to count the smallest annulus at the center of the thin-section as the first annulus. Following ASMFC’s instruction, we didn’t count the smallest annulus at the center as the first annulus in 2022.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers’ ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish,

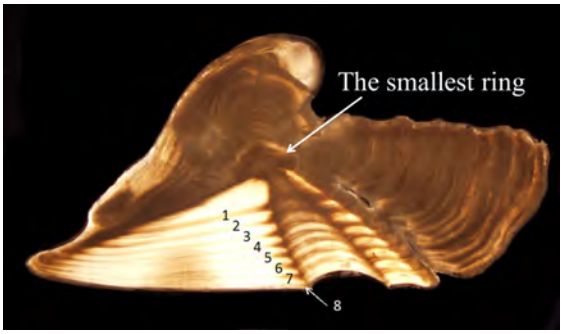


Figure 1.1: Otolith thin-sections of a 8 year-old Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 1.1).

1.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

1.3 RESULTS

1.3.1 Sample Size

We estimated a sample size of 374 Atlantic Croaker in 2022, ranging in length interval from 4 to 16 inches (Table 1.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for the major age of Age 4 to the CV of larger than 25% for the multiple minor ages (Table 1.2). In 2022, we aged 226 of 282 Croaker (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 163 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

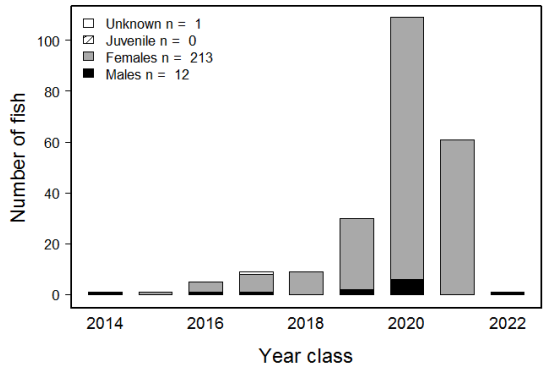


Figure 1.2: Year-class frequency distribution for Atlantic Croaker collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

1.3.2 Year Class

Of the 226 fish aged with otoliths, 9 age classes (0 to 8) were represented (Table 1.3). The average age was 2.2 years, and the standard deviation and standard error were 1.2 and 0.08, re-

spectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2014 to 2022 year-classes, with fish primarily from the year classes of 2020 and 2021 with 48.2% and 27%, respectively. The ratio of males to females was 1:17.75 in the sample collected (Figure 1.2).

1.3.3 Age-length Key (ALK)

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

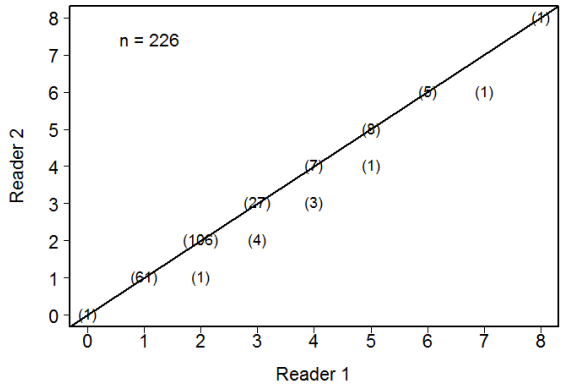


Figure 1.3: Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

1.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a CV of 0.26% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a CV of 0.26% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 95.58% and a CV of 1.1% (test of symmetry: $\chi^2 = 10$, $df = 5$, $P = 0.0752$) (Figure 1.3).

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

Table 1.1: Number of Atlantic Croaker collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
5 - 5.99	5	0	0	5
6 - 6.99	5	0	0	5
7 - 7.99	13	2	2	11
8 - 8.99	10	59	22	0
9 - 9.99	29	49	30	0
10 - 10.99	52	54	54	0
11 - 11.99	89	62	62	27
12 - 12.99	95	41	41	54
13 - 13.99	46	12	12	34
14 - 14.99	15	3	3	12
15 - 15.99	5	0	0	5
16 - 16.99	5	0	0	5
Totals	374	282	226	163

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Table 1.2: CV for each age estimated based on ageing the total of 374 Croaker in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Croaker collected from 2016 to 2020.

Age	CV	Percent
0	0.23	2.51
1	0.16	9.31
2	0.14	12.2
3	0.15	11.53
4	0.08	29.64
5	0.11	19.07
6	0.15	10.72
7	>0.25	2.73
8	>0.25	1.7
9	>0.25	0.59

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Table 1.3: The number of Atlantic Croaker assigned to each total length-at-age category for 226 fish sampled for otolith age determination in Virginia during 2022.

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
7 - 7.99	0	1	1	0	0	0	0	0	0	2
8 - 8.99	0	4	14	1	2	1	0	0	0	22
9 - 9.99	0	2	15	6	2	3	0	1	1	30
10 - 10.99	0	2	29	14	4	2	3	0	0	54
11 - 11.99	1	20	28	8	1	2	2	0	0	62
12 - 12.99	0	23	16	1	0	1	0	0	0	41
13 - 13.99	0	8	4	0	0	0	0	0	0	12
14 - 14.99	0	1	2	0	0	0	0	0	0	3
Totals	1	61	109	30	9	9	5	1	1	226

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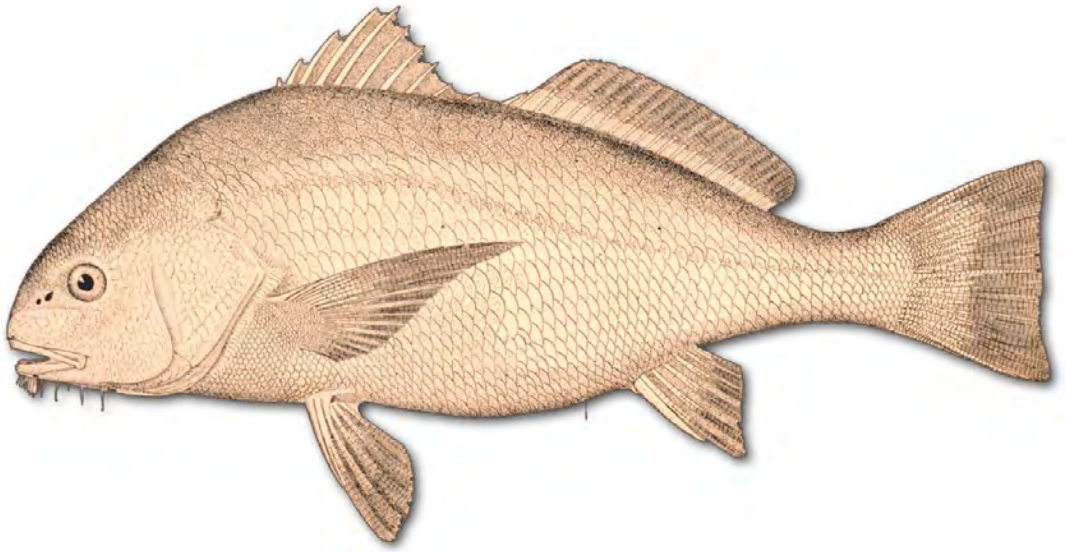
Table 1.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Croaker sampled for age determination in Virginia during 2022.

	Age								
Interval	0	1	2	3	4	5	6	7	8
7 - 7.99	0	0.5	0.5	0	0	0	0	0	0
8 - 8.99	0	0.18	0.64	0.05	0.09	0.05	0	0	0
9 - 9.99	0	0.07	0.5	0.2	0.07	0.1	0	0.03	0.03
10 - 10.99	0	0.04	0.54	0.26	0.07	0.04	0.06	0	0
11 - 11.99	0.02	0.32	0.45	0.13	0.02	0.03	0.03	0	0
12 - 12.99	0	0.56	0.39	0.02	0	0.02	0	0	0
13 - 13.99	0	0.67	0.33	0	0	0	0	0	0
14 - 14.99	0	0.33	0.67	0	0	0	0	0	0

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Chapter 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 107 Black Drum *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Black drum ages ranged from 0 to 49 years old with an average age of 8.8, a standard deviation of 8.9, and a standard error of 0.86. Twenty-five age classes (0, 3 to 8, 10 to 11, 13 to 18, 20 to 21, 23 to 26, 31 to 32, 36, and 49) were represented, comprising fish of the 1973, 1986, 1990 to 1991, 1996 to 1999, 2001 to 2002, 2004 to 2009, 2011 to 2012, 2014 to 2019, and 2022 year-classes. The sample was dominated by fish from the year-classes of 2018 and 2019 with 39.2% and 14%, respectively.

2.2 METHODS

2.2.1 Handling of Collections

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

2.2.2 Preparation

Otoliths were processed for age determination following the methods described in Bobko (1991) and Jones and Wells (1998). The otoliths were viewed by eye to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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

2.2.3 Readings

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

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

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

gin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

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

Wehn an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 2.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

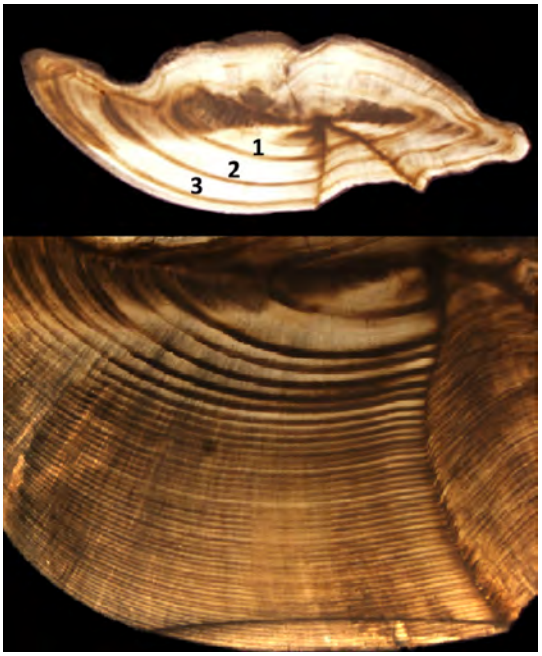


Figure 2.1: Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum.

2.2.4 Comparison Tests

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

sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2001 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

2.3 RESULTS

2.3.1 Year Class

We aged the otoliths of all the 107 Black Drum collected in 2022. Of the 107 fish aged, 25 age classes (0, 3 to 8, 10 to 11, 13 to 18, 20 to 21, 23 to 26, 31 to 32, 36, and 49) were represented (Table 2.1). The average age was 8.8 years, and the standard deviation and standard error were 8.9 and 0.86, respectively. Year-class data show that the fishery was comprised of 25 year-classes: fish from the 1973, 1986, 1990 to 1991, 1996 to 1999, 2001 to 2002, 2004 to 2009, 2011 to 2012, 2014 to 2019, and 2022 year-classes, with fish primarily from the year classes of 2018 and 2019 with 39.2% and 14%, respectively. The ratio of males to females was 1:1.41 in the sample collected (Figure 2.2).

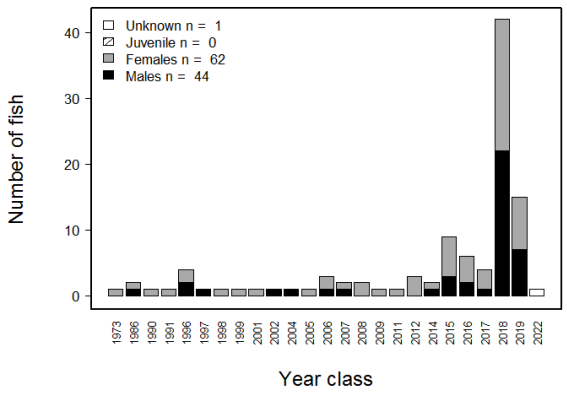


Figure 2.2: Year-class frequency distribution for Black Drum collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

2.3.2 Age-length Key (ALK)

We developed an age-length-key (Table 2.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

2.3.3 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a CV of 0.03% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 0.22% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.07% and a CV of 1.32% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$) (Figure 2.3).

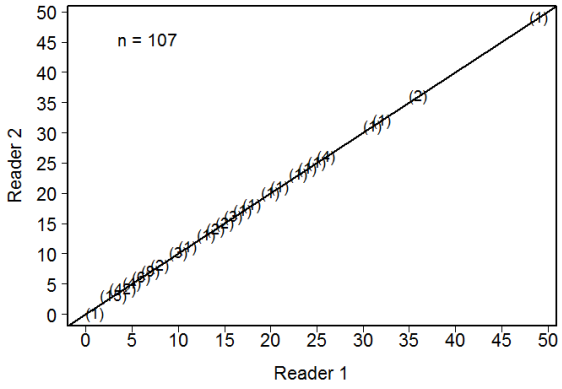


Figure 2.3: Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with ages of fish aged in 2001 with a CV of 0.09% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100%.

Table 2.1: The number of Black Drum assigned to each total length (inch)-at-age category for 107 fish sampled for otolith age determination in Virginia during 2022.

	Age																									
Interval	0	3	4	5	6	7	8	10	11	13	14	15	16	17	18	20	21	23	24	25	26	31	32	36	49	Totals
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
20 - 20.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
21 - 21.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
22 - 22.99	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
23 - 23.99	0	4	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
24 - 24.99	0	5	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
25 - 25.99	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
26 - 26.99	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
27 - 27.99	0	0	4	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
28 - 28.99	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
29 - 29.99	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
30 - 30.99	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
31 - 31.99	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
32 - 32.99	0	0	0	0	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
33 - 33.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
34 - 34.99	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
35 - 35.99	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
36 - 36.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
37 - 37.99	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
39 - 39.99	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
40 - 40.99	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	4
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	4
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2
Totals	1	15	42	4	6	9	2	3	1	1	2	2	3	1	1	1	1	1	1	1	4	1	1	2	1	107

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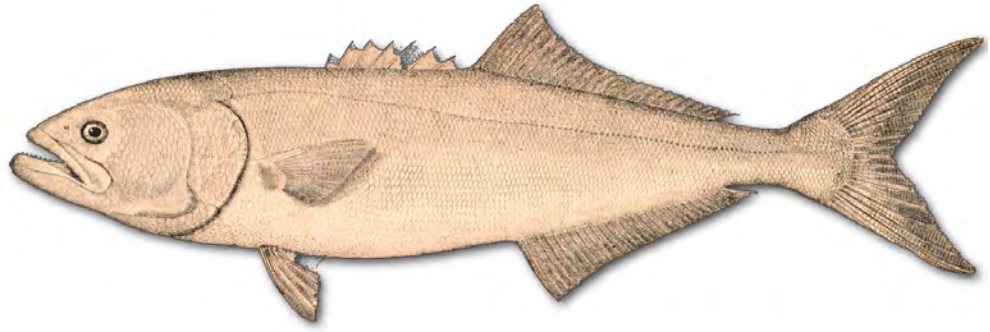
Table 2.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Black Drum sampled for age determination in Virginia during 2022.

	Age																								
Interval	0	3	4	5	6	7	8	10	11	13	14	15	16	17	18	20	21	23	24	25	26	31	32	36	49
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.75	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.36	0.55	0	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0.38	0.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0.12	0.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0.5	0.25	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.25	0.25	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0.33	0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0.2	0.4	0.2	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0.25	0.25	0.25	0	0.25	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0.25	0	0	0	0.5	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0

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Chapter 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 297 Bluefish *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Bluefish ages ranged from 0 to 8 years old with an average age of 2.6, a standard deviation of 1.9, and a standard error of 0.11. Nine age classes (0 to 8) were represented, comprising fish of the 2014 to 2022 year-classes. The sample was dominated by fish from the year-class of 2021 with 38.7%.

3.2 METHODS

3.2.1 Sample Size for Ageing

We estimated sample size for ageing Bluefish in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Bluefish in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Bluefish used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Bluefish collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022. Based on VMRC's request in 2010, we used 1-cm length interval for Bluefish, which differed from other species (1-inch).

3.2.2 Handling of Collections

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

3.2.3 Preparation

We used our bake and thin-section technique to process Bluefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination (Robillard et al. 2009). Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards. The otoliths were viewed under a stereo microscope to identify the location of the core. Then, the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of

the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broad and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

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

3.2.4 Readings

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

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

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

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

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 3.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section. 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 fol-

lowed outward from the sulcal groove towards the dorsal perimeter of the otolith. Often, but not always, the first year was associated with a very distinct crenellation on the dorsal surface and a prominent protrusion on the ventral surface. Both of these landmarks had a tendency to become less prominent in older fish.

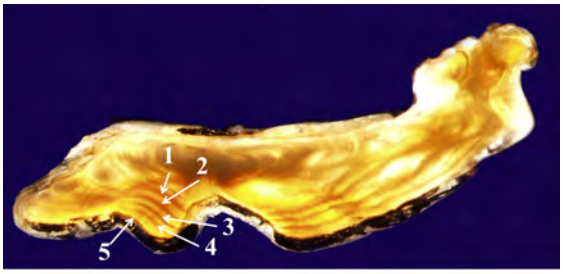


Figure 3.1: Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section

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

Even with the bake and thin-section technique, interpretation of the growth zones from the otoliths of young Bluefish was difficult. Rapid growth within the first year of life prevents a sharp delineation between opaque and translucent zones. When the exact location of the first year was not clearly evident, and the otolith had been sectioned accurately, a combination of surface landscape (1st year crenellation) and the position of the second annuli were used to help determine the position of the first annulus.

What appeared to be "double annuli" were occasionally observed in Bluefish 4-7 years of age and older. This double-annulus formation was typically characterized by distinct and separate annuli in extremely close proximity to each other. We do not know if the formation of these double annuli were two separate annuli, or in fact only one, but they seemed to occur during times of reduced growth after maturation. "Double annuli" were considered to be one annulus when both marks joined to form a central origin (the origin being the sulcal groove and the outer peripheral edge of the otolith). If these annuli did not meet to form a central origin they were considered two distinct annuli, and were counted as such.

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

3.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

3.3 RESULTS

3.3.1 Sample Size

We estimated a sample size of 421 Bluefish in 2022, ranging in length interval from 14 to 121 centimeters (Table 3.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 6% for Age 1 and 2 to the *CV* of larger than 25% for the multiple minor ages (Table 3.2). In 2022, we aged 297 of 376 Bluefish (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 160 fish, as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

3.3.2 Year Class

Of the 297 fish aged with otoliths, 9 age classes (0 to 8) were represented (Table 3.3). The average age was 2.6 years, and the standard deviation and standard error were 1.9 and 0.11, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2014 to 2022 year-classes, with fish primarily from the year class of 2021 with 38.7%. The ratio of males to females was 1:1.52 in the sample collected (Figure 3.2).

3.3.3 Age-length Key (ALK)

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

3.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a *CV* of 3.48% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a *CV* of 0.85% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.9% and a *CV* of 2.53% (test of symmetry: $\chi^2 = 11.49$, $df = 7$, $P = 0.1188$) (Figure 3.3).

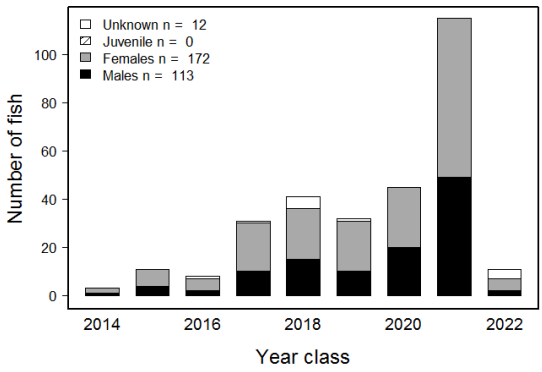


Figure 3.2: Year-class frequency distribution for Bluefish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

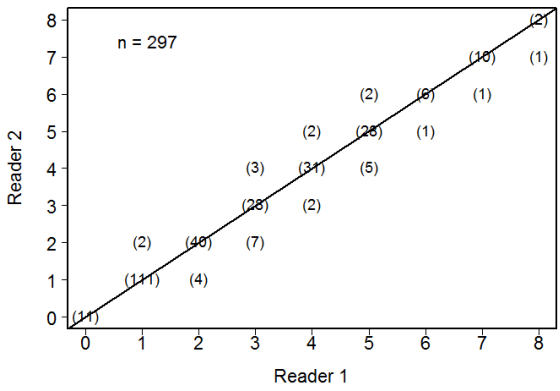


Figure 3.3: Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader.

Reader 1 had an agreement of 98% with ages of fish aged in 2000 with a CV of 2.83% (test of symmetry: $\chi^2 = 1,\ df = 1,\ P = 0.3173$). Reader 2 had an agreement of 98% with a CV of 0.94% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

Table 3.1: Number of Bluefish collected and aged in each 1-cm length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

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

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
58 - 58.99	5	2	2	3
59 - 59.99	5	4	4	1
60 - 60.99	5	6	6	0
61 - 61.99	5	4	4	1
62 - 62.99	5	3	3	2
63 - 63.99	5	0	0	5
64 - 64.99	5	4	4	1
65 - 65.99	5	2	2	3
66 - 66.99	5	1	1	4
67 - 67.99	5	5	5	0
68 - 68.99	5	9	9	0
69 - 69.99	5	7	7	0
70 - 70.99	5	7	7	0
71 - 71.99	5	6	6	0
72 - 72.99	5	11	11	0
73 - 73.99	5	1	1	4
74 - 74.99	5	3	3	2
75 - 75.99	5	2	2	3
76 - 76.99	5	3	3	2
77 - 77.99	5	0	0	5
78 - 78.99	5	6	6	0
79 - 79.99	5	6	6	0
80 - 80.99	5	4	4	1
81 - 81.99	5	3	3	2
82 - 82.99	5	3	3	2
83 - 83.99	5	2	2	3
84 - 84.99	5	4	4	1
85 - 85.99	5	2	2	3
86 - 86.99	5	4	4	1
87 - 87.99	5	0	0	5
88 - 88.99	5	2	2	3
89 - 89.99	5	1	1	4
90 - 90.99	5	3	3	2
91 - 91.99	5	1	1	4
92 - 92.99	5	0	0	5
93 - 93.99	5	0	0	5
94 - 94.99	5	0	0	5
95 - 95.99	5	0	0	5
96 - 96.99	5	0	0	5
121 - 121.99	5	0	0	5
Totals	421	376	297	160

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Table 3.2: CV for each age estimated based on ageing the total of 421 Bluefish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Bluefish collected from 2016 to 2020.

Age	CV	Percent
0	0.16	5.7
1	0.06	27.39
2	0.06	28.44
3	0.14	9.55
4	0.13	8.07
5	0.18	5.33
6	0.19	4.91
7	0.19	4.64
8	0.22	3.75
9	>0.25	1.27
10	>0.25	0.69
11	>0.25	0.16
12	>0.25	0.05
13	>0.25	0.05

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Table 3.3: The number of Bluefish assigned to each total length (cm)-at-age category for 297 fish sampled for otolith age determination in Virginia during 2022.

	Age								
Interval	0	1	2	3	4	5	6	7	8	Totals
20 - 20.99	1	0	0	0	0	0	0	0	0	1
21 - 21.99	1	0	0	0	0	0	0	0	0	1
23 - 23.99	2	0	0	0	0	0	0	0	0	2
24 - 24.99	4	2	0	0	0	0	0	0	0	6
25 - 25.99	3	3	0	0	0	0	0	0	0	6
26 - 26.99	0	6	0	0	0	0	0	0	0	6
27 - 27.99	0	6	0	0	0	0	0	0	0	6
28 - 28.99	0	6	0	0	0	0	0	0	0	6
29 - 29.99	0	6	0	0	0	0	0	0	0	6
30 - 30.99	0	5	1	0	0	0	0	0	0	6
31 - 31.99	0	6	0	0	0	0	0	0	0	6
32 - 32.99	0	6	0	0	0	0	0	0	0	6
33 - 33.99	0	6	0	0	0	0	0	0	0	6
34 - 34.99	0	6	0	0	0	0	0	0	0	6
35 - 35.99	0	6	0	0	0	0	0	0	0	6
36 - 36.99	0	6	0	0	0	0	0	0	0	6
37 - 37.99	0	5	1	0	0	0	0	0	0	6
38 - 38.99	0	6	0	0	0	0	0	0	0	6
39 - 39.99	0	5	1	0	0	0	0	0	0	6
40 - 40.99	0	6	0	0	0	0	0	0	0	6
41 - 41.99	0	4	2	0	0	0	0	0	0	6
42 - 42.99	0	5	1	0	0	0	0	0	0	6
43 - 43.99	0	4	1	1	0	0	0	0	0	6
44 - 44.99	0	3	3	1	0	0	0	0	0	7
45 - 45.99	0	3	1	0	0	0	0	0	0	4
46 - 46.99	0	2	3	1	0	0	0	0	0	6
47 - 47.99	0	1	3	4	0	0	0	0	0	8
48 - 48.99	0	0	3	0	0	0	0	0	0	3
49 - 49.99	0	1	3	1	0	0	0	0	0	5
50 - 50.99	0	0	3	3	0	0	0	0	0	6
51 - 51.99	0	0	3	1	0	0	0	0	0	4
52 - 52.99	0	0	3	0	0	0	0	0	0	3
53 - 53.99	0	0	1	0	0	0	0	0	0	1
55 - 55.99	0	0	2	1	0	0	0	0	0	3
57 - 57.99	0	0	0	1	1	0	0	0	0	2
58 - 58.99	0	0	1	1	0	0	0	0	0	2
59 - 59.99	0	0	0	4	0	0	0	0	0	4
60 - 60.99	0	0	0	4	2	0	0	0	0	6
61 - 61.99	0	0	0	3	1	0	0	0	0	4
62 - 62.99	0	0	1	2	0	0	0	0	0	3
64 - 64.99	0	0	1	0	2	1	0	0	0	4
65 - 65.99	0	0	1	0	1	0	0	0	0	2

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Table 3.3 (Continued)

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
66 - 66.99	0	0	0	0	1	0	0	0	0	1
67 - 67.99	0	0	1	0	3	1	0	0	0	5
68 - 68.99	0	0	1	3	3	2	0	0	0	9
69 - 69.99	0	0	2	0	4	1	0	0	0	7
70 - 70.99	0	0	1	0	5	1	0	0	0	7
71 - 71.99	0	0	0	1	5	0	0	0	0	6
72 - 72.99	0	0	1	0	7	3	0	0	0	11
73 - 73.99	0	0	0	0	1	0	0	0	0	1
74 - 74.99	0	0	0	0	2	1	0	0	0	3
75 - 75.99	0	0	0	0	1	1	0	0	0	2
76 - 76.99	0	0	0	0	1	2	0	0	0	3
78 - 78.99	0	0	0	0	0	3	3	0	0	6
79 - 79.99	0	0	0	0	0	5	1	0	0	6
80 - 80.99	0	0	0	0	0	3	1	0	0	4
81 - 81.99	0	0	0	0	0	1	1	0	1	3
82 - 82.99	0	0	0	0	1	2	0	0	0	3
83 - 83.99	0	0	0	0	0	1	0	1	0	2
84 - 84.99	0	0	0	0	0	1	1	2	0	4
85 - 85.99	0	0	0	0	0	1	1	0	0	2
86 - 86.99	0	0	0	0	0	1	0	3	0	4
88 - 88.99	0	0	0	0	0	0	0	0	2	2
89 - 89.99	0	0	0	0	0	0	0	1	0	1
90 - 90.99	0	0	0	0	0	0	0	3	0	3
91 - 91.99	0	0	0	0	0	0	0	1	0	1
Totals	11	115	45	32	41	31	8	11	3	297

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Table 3.4: Age-Length key, as proportion-at-age in each 1-cm length interval, based on otolith ages for Bluefish sampled for age determination in Virginia during 2022.

	Age								
Interval	0	1	2	3	4	5	6	7	8
20 - 20.99	1	0	0	0	0	0	0	0	0
21 - 21.99	1	0	0	0	0	0	0	0	0
23 - 23.99	1	0	0	0	0	0	0	0	0
24 - 24.99	0.67	0.33	0	0	0	0	0	0	0
25 - 25.99	0.5	0.5	0	0	0	0	0	0	0
26 - 26.99	0	1	0	0	0	0	0	0	0
27 - 27.99	0	1	0	0	0	0	0	0	0
28 - 28.99	0	1	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	0	0	0	0
30 - 30.99	0	0.83	0.17	0	0	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0	0	0
32 - 32.99	0	1	0	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0	0
34 - 34.99	0	1	0	0	0	0	0	0	0
35 - 35.99	0	1	0	0	0	0	0	0	0
36 - 36.99	0	1	0	0	0	0	0	0	0
37 - 37.99	0	0.83	0.17	0	0	0	0	0	0
38 - 38.99	0	1	0	0	0	0	0	0	0
39 - 39.99	0	0.83	0.17	0	0	0	0	0	0
40 - 40.99	0	1	0	0	0	0	0	0	0
41 - 41.99	0	0.67	0.33	0	0	0	0	0	0
42 - 42.99	0	0.83	0.17	0	0	0	0	0	0
43 - 43.99	0	0.67	0.17	0.17	0	0	0	0	0
44 - 44.99	0	0.43	0.43	0.14	0	0	0	0	0
45 - 45.99	0	0.75	0.25	0	0	0	0	0	0
46 - 46.99	0	0.33	0.5	0.17	0	0	0	0	0
47 - 47.99	0	0.12	0.38	0.5	0	0	0	0	0
48 - 48.99	0	0	1	0	0	0	0	0	0
49 - 49.99	0	0.2	0.6	0.2	0	0	0	0	0
50 - 50.99	0	0	0.5	0.5	0	0	0	0	0
51 - 51.99	0	0	0.75	0.25	0	0	0	0	0
52 - 52.99	0	0	1	0	0	0	0	0	0
53 - 53.99	0	0	1	0	0	0	0	0	0
55 - 55.99	0	0	0.67	0.33	0	0	0	0	0
57 - 57.99	0	0	0	0.5	0.5	0	0	0	0
58 - 58.99	0	0	0.5	0.5	0	0	0	0	0
59 - 59.99	0	0	0	1	0	0	0	0	0
60 - 60.99	0	0	0	0.67	0.33	0	0	0	0
61 - 61.99	0	0	0	0.75	0.25	0	0	0	0
62 - 62.99	0	0	0.33	0.67	0	0	0	0	0
64 - 64.99	0	0	0.25	0	0.5	0.25	0	0	0
65 - 65.99	0	0	0.5	0	0.5	0	0	0	0

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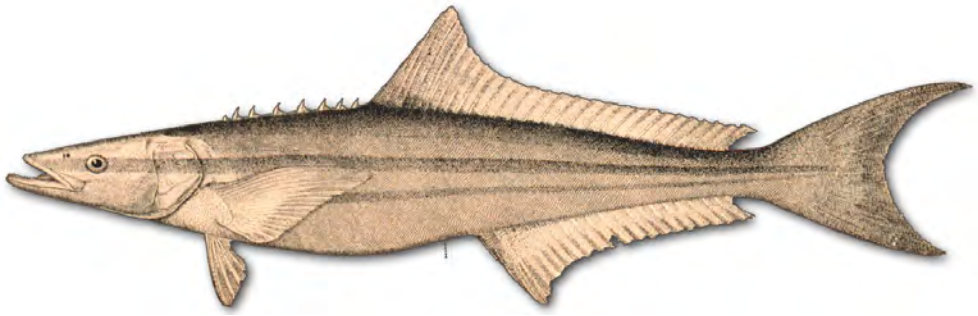
Table 3.4 (Continued)

	Age								
Interval	0	1	2	3	4	5	6	7	8
66 - 66.99	0	0	0	0	1	0	0	0	0
67 - 67.99	0	0	0.2	0	0.6	0.2	0	0	0
68 - 68.99	0	0	0.11	0.33	0.33	0.22	0	0	0
69 - 69.99	0	0	0.29	0	0.57	0.14	0	0	0
70 - 70.99	0	0	0.14	0	0.71	0.14	0	0	0
71 - 71.99	0	0	0	0.17	0.83	0	0	0	0
72 - 72.99	0	0	0.09	0	0.64	0.27	0	0	0
73 - 73.99	0	0	0	0	1	0	0	0	0
74 - 74.99	0	0	0	0	0.67	0.33	0	0	0
75 - 75.99	0	0	0	0	0.5	0.5	0	0	0
76 - 76.99	0	0	0	0	0.33	0.67	0	0	0
78 - 78.99	0	0	0	0	0	0.5	0.5	0	0
79 - 79.99	0	0	0	0	0	0.83	0.17	0	0
80 - 80.99	0	0	0	0	0	0.75	0.25	0	0
81 - 81.99	0	0	0	0	0	0.33	0.33	0	0.33
82 - 82.99	0	0	0	0	0.33	0.67	0	0	0
83 - 83.99	0	0	0	0	0	0.5	0	0.5	0
84 - 84.99	0	0	0	0	0	0.25	0.25	0.5	0
85 - 85.99	0	0	0	0	0	0.5	0.5	0	0
86 - 86.99	0	0	0	0	0	0.25	0	0.75	0
88 - 88.99	0	0	0	0	0	0	0	0	1
89 - 89.99	0	0	0	0	0	0	0	1	0
90 - 90.99	0	0	0	0	0	0	0	1	0
91 - 91.99	0	0	0	0	0	0	0	1	0

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Chapter 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 327 Cobia *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Cobia ages ranged from 2 to 10 years old with an average age of 5.3, a standard deviation of 1.6, and a standard error of 0.09. Eight age classes (2 to 8, and 10) were represented, comprising fish of the 2012, and 2014 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2016 and 2018 with 29% and 37.3%, respectively.

4.2 METHODS

4.2.1 Handling of Collections

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

4.2.2 Preparation

Otoliths were processed for age determination. The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

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

4.2.3 Readings

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

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

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

is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Cobia otolith annulus formation occurs between June and July (Richards 1967 and modified by CQFE/ODU). A Cobia with five visible annuli could be assigned an age of 5 or 6 depending on its capture month and margin code. When its margin code is "1", it is Age 5 no matter when it is captured. When it is captured after July and before January, it is Age 5 no matter what its margin code is. When it is captured after December and before June and its margin code is not "1", it is Age 6 (5 + 1 = 6). When it is captured between June and July, it is Age 5 when its margin code is "2" but Age 6 (5 + 1 = 6) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 4.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

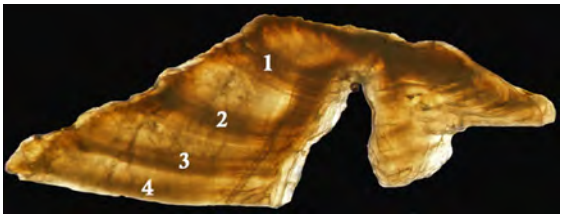


Figure 4.1: Otolith thin-section of a 4 year-old Cobia.

4.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

4.3 RESULTS

4.3.1 Year Class

We aged the otoliths of all the 327 Cobia collected in 2022. Of the 327 fish aged, 8 age classes (2 to 8, and 10) were represented (Table 4.1). The average age was 5.3 years, and the standard deviation and standard error were 1.6 and 0.09, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2012, and 2014 to 2020 year-classes, with fish primarily from the year classes of 2016 and 2018 with 29% and 37.3%, respectively. The ratio of males to females was 1:1.71 in the sample collected (Figure 4.2).

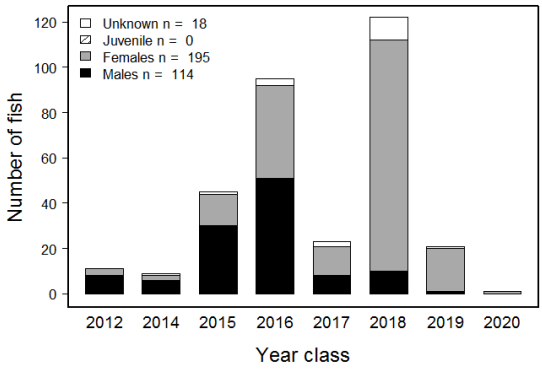


Figure 4.2: Year-class frequency distribution for Cobia collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

4.3.2 Age-length Key (ALK)

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

4.3.3 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a CV of 0.89% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 0.44% (test of symmetry: $\chi^2 = 2$, $df = 1$, $P = 0.1573$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 96.64% and a CV of 0.49% (test of symmetry: $\chi^2 = 9$, $df = 9$, $P = 0.4373$) (Figure 4.3).

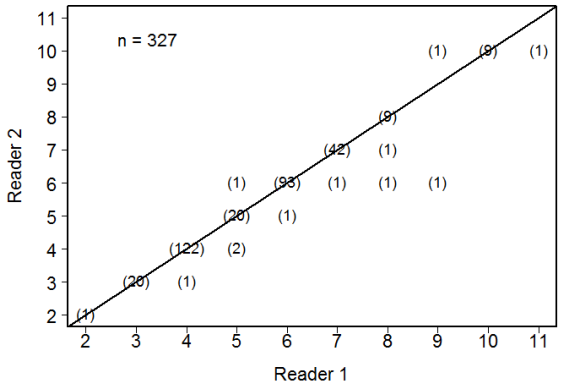


Figure 4.3: Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 80% with ages of fish aged in 2000 with a CV of 2.07% (test of symmetry: $\chi^2 = 8$, $df = 5$, $P = 0.1562$). Reader 2 had an agreement of 92% with a CV of 0.55% (test of symmetry: $\chi^2 = 2$, $df = 3$, $P = 0.5724$).

Table 4.1: The number of Cobia assigned to each total length (inch)-at-age category for 327 fish sampled for otolith age determination in Virginia during 2022.

	Age								
Interval	2	3	4	5	6	7	8	10	Totals
36 - 36.99	1	1	1	0	0	0	0	0	3
37 - 37.99	0	2	3	1	2	1	0	0	9
38 - 38.99	0	5	5	0	1	0	0	0	11
39 - 39.99	0	7	14	2	8	0	0	0	31
40 - 40.99	0	2	14	1	9	3	0	0	29
41 - 41.99	0	2	18	1	15	4	2	0	42
42 - 42.99	0	2	16	5	8	9	0	0	40
43 - 43.99	0	0	17	1	5	4	3	1	31
44 - 44.99	0	0	17	1	9	5	1	1	34
45 - 45.99	0	0	10	2	5	6	1	4	28
46 - 46.99	0	0	4	2	5	0	1	2	14
47 - 47.99	0	0	1	5	2	3	0	1	12
48 - 48.99	0	0	0	0	10	0	0	1	11
49 - 49.99	0	0	2	0	7	0	0	0	9
50 - 50.99	0	0	0	1	5	4	0	0	10
51 - 51.99	0	0	0	1	1	2	0	0	4
52 - 52.99	0	0	0	0	1	1	0	0	2
53 - 53.99	0	0	0	0	1	1	1	0	3
54 - 54.99	0	0	0	0	1	2	0	0	3
55 - 55.99	0	0	0	0	0	0	0	1	1
Totals	1	21	122	23	95	45	9	11	327

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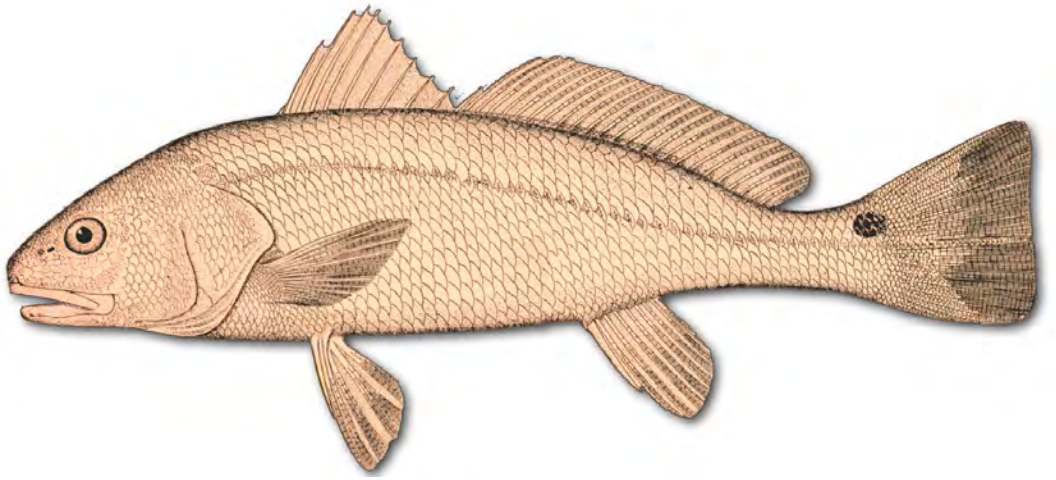
Table 4.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Cobia sampled for age determination in Virginia during 2022.

	Age							
Interval	2	3	4	5	6	7	8	10
36 - 36.99	0.33	0.33	0.33	0	0	0	0	0
37 - 37.99	0	0.22	0.33	0.11	0.22	0.11	0	0
38 - 38.99	0	0.45	0.45	0	0.09	0	0	0
39 - 39.99	0	0.23	0.45	0.06	0.26	0	0	0
40 - 40.99	0	0.07	0.48	0.03	0.31	0.1	0	0
41 - 41.99	0	0.05	0.43	0.02	0.36	0.1	0.05	0
42 - 42.99	0	0.05	0.4	0.12	0.2	0.22	0	0
43 - 43.99	0	0	0.55	0.03	0.16	0.13	0.1	0.03
44 - 44.99	0	0	0.5	0.03	0.26	0.15	0.03	0.03
45 - 45.99	0	0	0.36	0.07	0.18	0.21	0.04	0.14
46 - 46.99	0	0	0.29	0.14	0.36	0	0.07	0.14
47 - 47.99	0	0	0.08	0.42	0.17	0.25	0	0.08
48 - 48.99	0	0	0	0	0.91	0	0	0.09
49 - 49.99	0	0	0.22	0	0.78	0	0	0
50 - 50.99	0	0	0	0.1	0.5	0.4	0	0
51 - 51.99	0	0	0	0.25	0.25	0.5	0	0
52 - 52.99	0	0	0	0	0.5	0.5	0	0
53 - 53.99	0	0	0	0	0.33	0.33	0.33	0
54 - 54.99	0	0	0	0	0.33	0.67	0	0
55 - 55.99	0	0	0	0	0	0	0	1

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Chapter 5

RED DRUM *Sciaenops ocellatus*



5.1 INTRODUCTION

We aged a total of 93 Red Drum *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Red drum ages ranged from 0 to 49 years old with an average age of 9, a standard deviation of 13.8, and a standard error of 1.43. Nineteen age classes (0 to 3, 10, 13, 15 to 17, 23 to 25, 30 to 31, 36, 38, 41, 47, and 49) were represented, comprising fish of the 1973, 1975, 1981, 1984, 1986, 1991 to 1992, 1997 to 1999, 2005 to 2007, 2009, 2012, and 2019 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2020 and 2021 with 33.3% and 31.2%, respectively.

5.2 METHODS

5.2.1 Handling of Collections

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

5.2.2 Preparation

Otoliths were processed for age determination following the methods described in Ross et al. (1995) and Jones and Wells (1998) for Red Drum. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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

5.2.3 Readings

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

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

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish

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

For example, Red Drum otolith annulus formation occurs between March and July (Ross et al. 1995 and modified by CQFE/ODU). A Red Drum with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after July and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before March and its margin code is not "1", it is Age 3 (2 + 1 = 3). When it is captured between March and July, it is Age 2 when its margin code is "2" but Age 3 (2 + 1 = 3) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 5.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section. However, due to discrepancy on identification of the first annulus of Red Drum among Atlantic states, ASMFC has decided not to count the smallest annulus at the center of the thin-section as the first annulus. Following ASMFC's instruction, we didn't count the smallest annulus at the center as the first annulus in 2022.

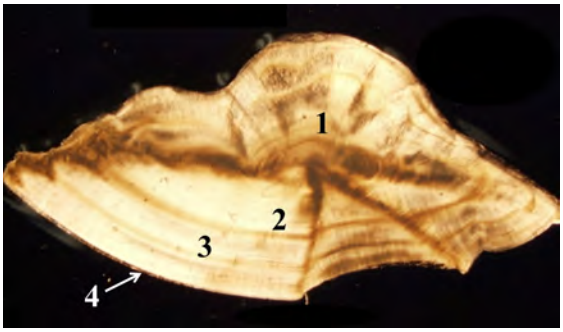


Figure 5.1: Otolith thin-section of a 3 year-old Red Drum with the last annulus on the edge of the thin-section

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 4.1).

5.2.4 Comparison Tests

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

the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

5.3 RESULTS

5.3.1 Year Class

We aged the otoliths of all the 93 Red Drum collected in 2022. Of the 93 fish aged, 93 fish aged with otoliths, 19 age classes (0 to 3, 10, 13, 15 to 17, 23 to 25, 30 to 31, 36, 38, 41, 47, and 49) were represented (Table 5.1). The average age was 9 years, and the standard deviation and standard error were 13.8 and 1.43, respectively. Year-class data show that the fishery was comprised of 19 year-classes: fish from the 1973, 1975, 1981, 1984, 1986, 1991 to 1992, 1997 to 1999, 2005 to 2007, 2009, 2012, and 2019 to 2022 year-classes, with fish primarily from the year classes of 2020 and 2021 with 33.3% and 31.2%, respectively. The ratio of males to females was 1:0.7 in the sample collected (Figure 5.2).

5.3.2 Age-length Key (ALK)

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

5.3.3 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a CV of 0.32% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$),

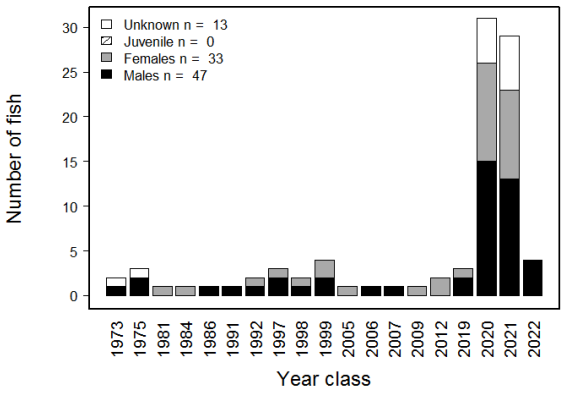


Figure 5.2: Year-class frequency distribution for Red Drum collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96%and a CV of 0.64% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.32% and a CV of 1.02% (test of symmetry: $\chi^2 = 9$, $df = 8$, $P = 0.3423$) (Figure 5.3).

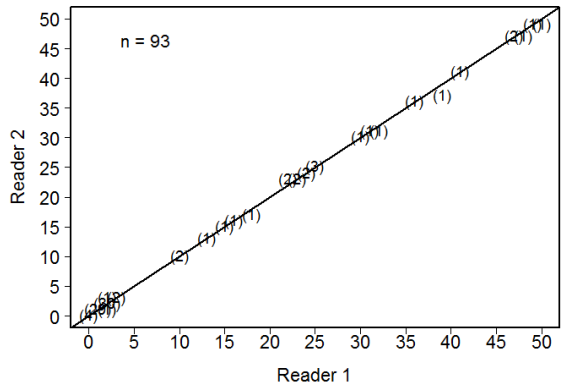


Figure 5.3: Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages

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

Table 5.1: The number of Red Drum assigned to each total length (inch)-at-age category for 93 fish sampled for otolith age determination in Virginia during 2022.

	Age																			
Interval	0	1	2	3	10	13	15	16	17	23	24	25	30	31	36	38	41	47	49	Totals
18 - 18.99	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
19 - 19.99	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
20 - 20.99	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
21 - 21.99	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
22 - 22.99	0	5	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
23 - 23.99	0	4	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
24 - 24.99	0	8	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
25 - 25.99	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
26 - 26.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
28 - 28.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
29 - 29.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
42 - 42.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
43 - 43.99	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
44 - 44.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
45 - 45.99	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	3
46 - 46.99	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	3
47 - 47.99	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	3
48 - 48.99	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2
49 - 49.99	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0	1	0	0	1	6
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2
51 - 51.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
52 - 52.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
53 - 53.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2
Totals	4	29	31	3	2	1	1	1	1	4	2	3	2	1	1	1	1	3	2	93

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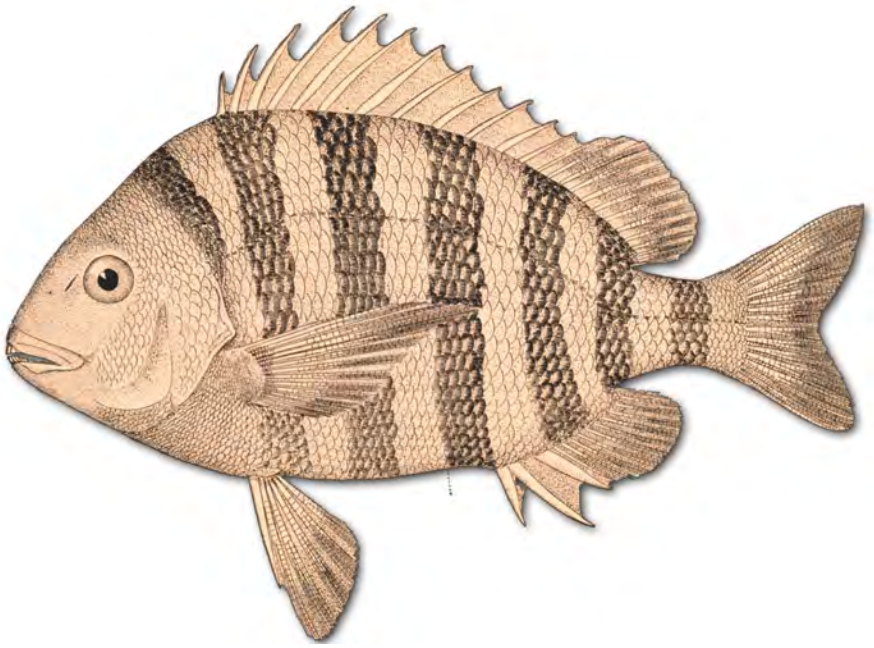
Table 5.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Red Drum sampled for age determination in Virginia during 2022.

	Age																		
Interval	0	1	2	3	10	13	15	16	17	23	24	25	30	31	36	38	41	47	49
18 - 18.99	0.5	0.38	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.75	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.45	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.4	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0.44	0.5	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44 - 44.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0.33	0	0	0	0	0	0	0.33	0	0	0	0.33	0
46 - 46.99	0	0	0	0	0	0.33	0	0	0	0	0	0.33	0	0	0.33	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0.33	0	0	0	0.33	0	0	0	0	0	0.33	0
48 - 48.99	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0.17	0.33	0	0.17	0	0	0.17	0	0	0.17
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0
51 - 51.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
52 - 52.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
53 - 53.99	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0.5

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Chapter 6

SHEEPSHEAD *Archosargus probatocephalus*



6.1 INTRODUCTION

We aged a total of 469 Sheepshead *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Sheepshead ages ranged from 1 to 40 years old with an average age of 12.2, a standard deviation of 7.6, and a standard error of 0.35. Thirty-two age classes (1 to 19, 21 to 32, and 40) were represented, comprising fish of the 1982, 1990 to 2001, and 2003 to 2021 year-classes. The sample was dominated by fish from the year-classes of 2011 and 2016 with 15.3% and 14.7%, respectively.

6.2 METHODS

6.2.1 Handling of Collections

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

6.2.2 Preparation

Otoliths were processed for age determination following the methods described in Ballenger (2011). The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

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

6.2.3 Readings

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

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

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its

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

For example, Sheepshead otolith annulus formation occurs between May and July (Ballenger 2011 and modified by CQFE/ODU). A Sheepshead with nine visible annuli could be assigned an age of 9 or 10 depending on its capture month and margin code. When its margin code is "1", it is Age 9 no matter when it is captured. When it is captured after July and before January, it is Age 9 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 10 (9 + 1 = 10). When it is captured between May and July, it is Age 9 when its margin code is "2" but Age 10 (9 + 1 = 10) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 6.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.



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

6.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2008 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

6.3 RESULTS

6.3.1 Year Class

We aged the otoliths of all the 469 Sheepshead collected in 2022. Of the 469 fish aged, 32 age classes (1 to 19, 21 to 32, and 40) were represented (Table 6.1). The average age was 12.2 years, and the standard deviation and standard error were 7.6 and 0.35, respectively. Year-class data show that the fishery was comprised of 32 year-classes: fish from the 1982, 1990 to 2001, and 2003 to 2021 year-classes, with fish primarily from the year classes of 2011 and 2016 with 15.3% and 14.7%, respectively. The ratio of males to females was 1:1.42 in the sample collected (Figure 6.2).

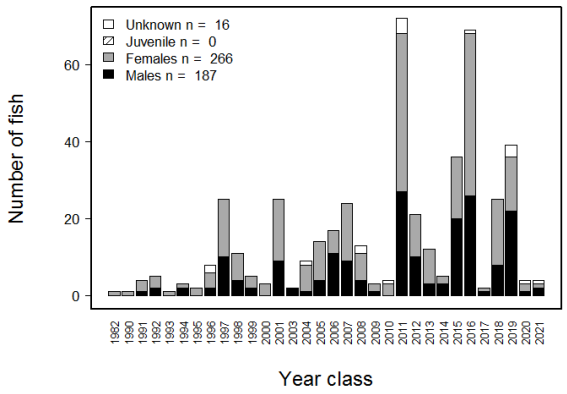


Figure 6.2: Year-class frequency distribution for Sheepshead collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

6.3.2 Age-length Key (ALK)

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

6.3.3 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a CV of 0.8% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a CV of 0.62% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.55% and a CV of 0.65% (test of symmetry: $\chi^2 = 29.33$, $df = 26$, $P = 0.2961$) (Figure 6.3).

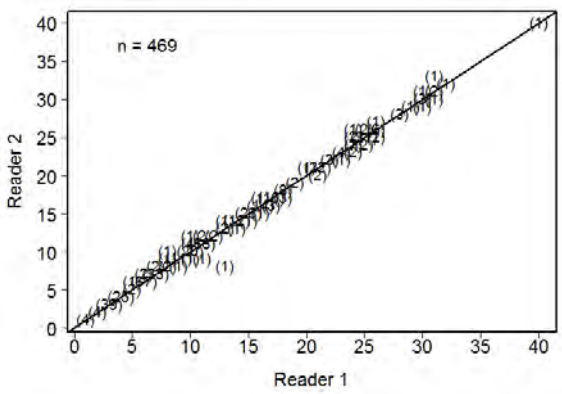


Figure 6.3: Between-reader comparison of otolith age estimates for Sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with ages of fish aged in 2008 with a CV of 0.57% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100% .

Table 6.1: The number of sheepshead assigned to each total length (inch)-at-age category for 469 fish sampled for otolith age determination in Virginia during 2022.

	Age																															
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31	32	40	Totals
9 - 9.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10 - 10.99	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
11 - 11.99	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
12 - 12.99	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
13 - 13.99	0	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
14 - 14.99	0	0	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
15 - 15.99	0	0	11	1	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
16 - 16.99	0	0	8	6	0	4	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22
17 - 17.99	0	0	4	6	0	7	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
18 - 18.99	0	0	0	8	1	17	4	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
19 - 19.99	0	0	0	1	1	18	5	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27
20 - 20.99	0	0	0	0	0	13	9	2	5	2	5	2	0	4	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45
21 - 21.99	0	0	0	0	0	7	8	0	6	10	19	1	2	2	5	4	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	69
22 - 22.99	0	0	0	0	0	1	4	2	1	6	28	0	1	3	7	5	3	2	0	9	1	2	3	8	3	0	1	0	1	1	1	0	93
23 - 23.99	0	0	0	0	0	0	0	0	0	1	13	1	0	3	8	5	9	6	1	10	1	3	4	4	3	2	1	0	1	1	0	1	78
24 - 24.99	0	0	0	0	0	0	0	0	0	0	3	0	0	1	2	1	1	0	1	6	0	0	3	9	2	0	1	0	1	1	0	0	32
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	3	0	0	0	0	1	1	0	0	8
Totals	4	4	39	25	2	69	36	5	12	21	72	4	3	13	24	17	14	9	2	25	3	5	11	25	8	2	3	1	5	4	1	1	469

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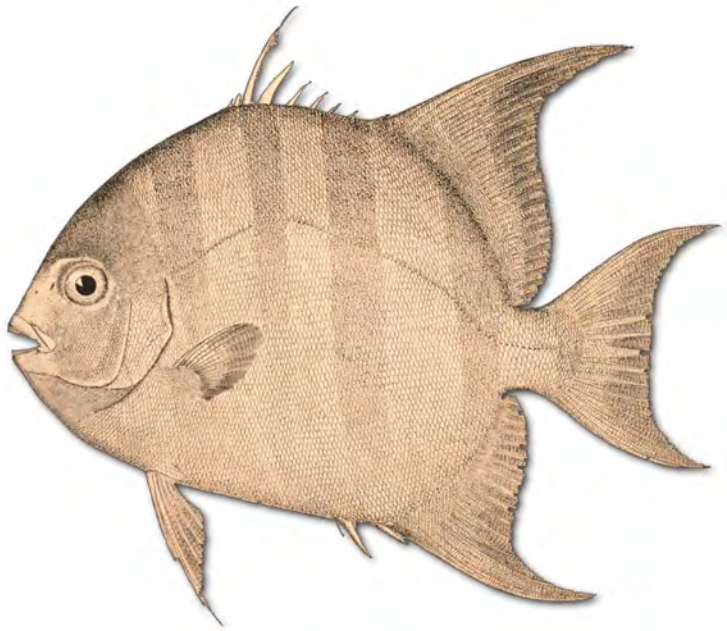
Table 6.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Sheepshead sampled for age determination in Virginia during 2022.

	Age																														
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31	32	40
9 - 9.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11 - 11.99	0.4	0.2	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 - 12.99	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 - 13.99	0	0	0.86	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0	0.75	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 - 15.99	0	0	0.69	0.06	0	0.12	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0	0	0.36	0.27	0	0.18	0.14	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0	0.21	0.32	0	0.37	0.05	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0	0	0	0.24	0.03	0.52	0.12	0.03	0	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0	0.04	0.04	0.67	0.19	0	0	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0	0	0.29	0.2	0.04	0.11	0.04	0.11	0.04	0	0.09	0.02	0.02	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0	0.1	0.12	0	0.09	0.14	0.28	0.01	0.03	0.03	0.07	0.06	0.01	0	0	0	0	0	0.01	0.01	0	0	0	0.01	0.01	0	0	0
22 - 22.99	0	0	0	0	0	0.01	0.04	0.02	0.01	0.06	0.3	0	0.01	0.03	0.08	0.05	0.03	0.02	0	0.1	0.01	0.02	0.03	0.09	0.03	0	0.01	0	0.01	0.01	0.01	0
23 - 23.99	0	0	0	0	0	0	0	0	0	0.01	0.17	0.01	0	0.04	0.1	0.06	0.12	0.08	0.01	0.13	0.01	0.04	0.05	0.05	0.04	0.03	0.01	0	0.01	0.01	0	0.01
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0.09	0	0	0.03	0.06	0.03	0.03	0	0.03	0.19	0	0	0.09	0.28	0.06	0	0.03	0	0.03	0.03	0	0
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.12	0	0	0	0	0.12	0	0	0.38	0	0	0	0	0.12	0.12	0	0

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Chapter 7

ATLANTIC SPADEFISH
Chaetodipterus faber



7.1 INTRODUCTION

We aged a total of 249 Spadefish *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Spadefish ages ranged from 0 to 8 years old with an average age of 2.9, a standard deviation of 1.6, and a standard error of 0.1. Nine age classes (0 to 8) were represented, comprising fish of the 2014 to 2022 year-classes. The sample was dominated by fish from the year-class of 2020 with 32.9%.

7.2 METHODS

7.2.1 Sample Size for Ageing

We estimated sample size for ageing Spadefish in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Spadefish in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spadefish used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Spadefish collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

7.2.2 Handling of Collections

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

7.2.3 Preparation

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the

otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distored winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

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

7.2.4 Readings

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

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

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

For example, Spadefish otolith annulus formation occurs between January and July (Hayse 1987 and modified by CQFE/ODU). A Spadefish with three visible annuli could be assigned an age of 3 or 4 depending on its capture month and margin code. When its margin code is "1", it is Age 3 no matter when it is captured. When it is captured after July and before January, it is Age 3 no matter what its margin code is. When it is captured between January and July, it is Age 3 when its margin code is "2" but Age 4 (3 + 1 = 4) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 7.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the read-

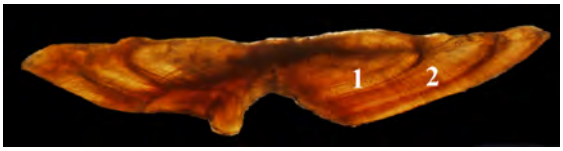


Figure 7.1: Otolith thin-section of a 2 year-old Spadefish

ers' 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

7.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

7.3 RESULTS

7.3.1 Sample Size

We estimated a sample size of 357 Spadefish in 2022, ranging in length interval from 3 to 21 inches (Table 7.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 7% for Age 2 to the CV of larger than 25% for the multiple minor ages (Table 7.2). In 2022, we Spadefishaged all 249 collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 121 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

7.3.2 Year Class

Of the 249 fish aged with otoliths, 9 age classes (0 to 8) were represented (Table 7.3). The average age was 2.9 years, and the standard deviation and standard error were 1.6 and 0.1, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2014 to 2022 year-classes, with fish primarily from the year class of 2020 with 32.9%. The ratio of males to females was 1:1.08 in the sample collected (Figure 7.2).

7.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 7.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

7.3.4 Reading Precision

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

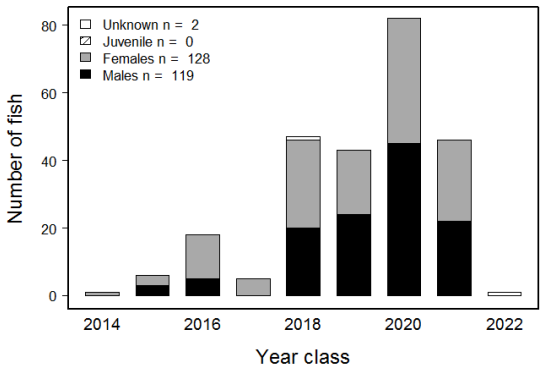


Figure 7.2: Year-class frequency distribution for Spadefish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

1 with an agreement of 94% and a CV of 3.45% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 0.57% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.76% and a CV of 2.32% (test of symmetry: $\chi^2 = 16.5$, $df = 9$, $P = 0.0571$) (Figure 7.3).

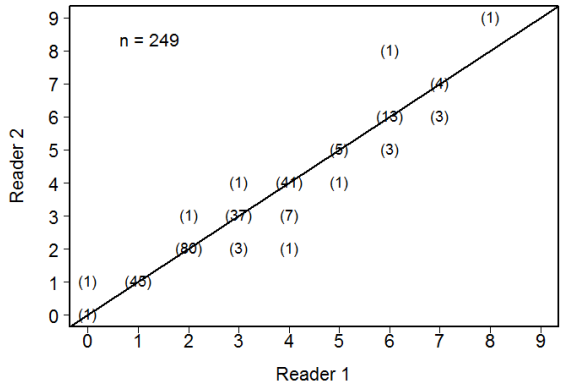


Figure 7.3: Between-reader comparison of otolith age estimates for Spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader.

Reader 1 had an agreement of 90% with ages of fish aged in 2003 with a CV of 1.56% (test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$). Reader 2 had an agreement of 82% with a CV of 2.15% (test of symmetry: $\chi^2 = 9$, $df = 9$, $P = 0.4373$).

Table 7.1: Number of Atlantic Spadefish collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
3 - 3.99	5	1	1	4
4 - 4.99	7	0	0	7
5 - 5.99	13	26	26	0
6 - 6.99	40	35	35	5
7 - 7.99	47	28	28	19
8 - 8.99	35	22	22	13
9 - 9.99	23	14	14	9
10 - 10.99	17	15	15	2
11 - 11.99	17	17	17	0
12 - 12.99	26	10	10	16
13 - 13.99	23	19	19	4
14 - 14.99	20	12	12	8
15 - 15.99	17	14	14	3
16 - 16.99	15	7	7	8
17 - 17.99	21	8	8	13
18 - 18.99	12	9	9	3
19 - 19.99	9	5	5	4
20 - 20.99	5	5	5	0
21 - 21.99	5	2	2	3
Totals	357	249	249	121

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Table 7.2: CV for each age estimated based on ageing the total of 357 Spadefish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spadefish collected from 2016 to 2020.

Age	CV	Percent
0	>0.25	0.48
1	0.12	10.46
2	0.07	27.29
3	0.1	21.47
4	0.1	21.19
5	0.14	11.08
6	0.21	5.4
7	>0.25	1.59
8	>0.25	0.76
9	>0.25	0.21
10	>0.25	0.07

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Table 7.3: The number of Atlantic Spadefish assigned to each total length-at-age category for 249 fish sampled for otolith age determination in Virginia during 2022.

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
3 - 3.99	1	0	0	0	0	0	0	0	0	1
5 - 5.99	0	20	6	0	0	0	0	0	0	26
6 - 6.99	0	16	19	0	0	0	0	0	0	35
7 - 7.99	0	8	18	2	0	0	0	0	0	28
8 - 8.99	0	2	14	6	0	0	0	0	0	22
9 - 9.99	0	0	12	2	0	0	0	0	0	14
10 - 10.99	0	0	7	7	1	0	0	0	0	15
11 - 11.99	0	0	6	11	0	0	0	0	0	17
12 - 12.99	0	0	0	4	6	0	0	0	0	10
13 - 13.99	0	0	0	7	12	0	0	0	0	19
14 - 14.99	0	0	0	4	7	0	1	0	0	12
15 - 15.99	0	0	0	0	11	2	1	0	0	14
16 - 16.99	0	0	0	0	5	0	2	0	0	7
17 - 17.99	0	0	0	0	2	0	4	2	0	8
18 - 18.99	0	0	0	0	1	2	6	0	0	9
19 - 19.99	0	0	0	0	1	0	1	3	0	5
20 - 20.99	0	0	0	0	1	0	2	1	1	5
21 - 21.99	0	0	0	0	0	1	1	0	0	2
Totals	1	46	82	43	47	5	18	6	1	249

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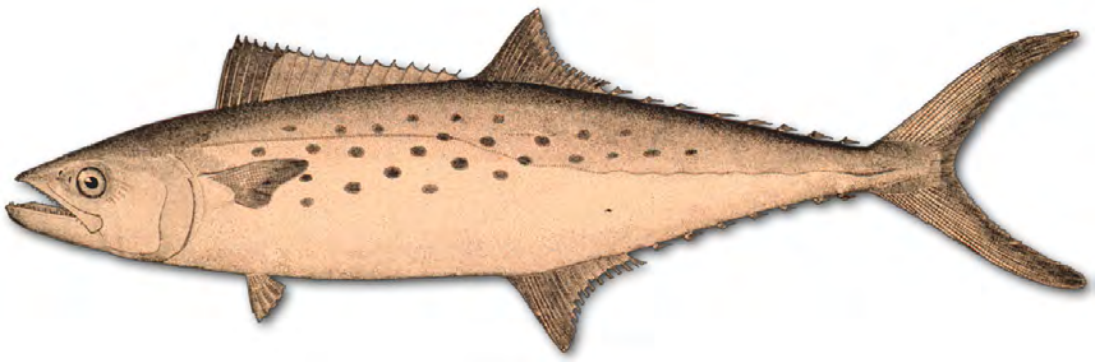
Table 7.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Spadefish sampled for age determination in Virginia during 2022.

	Age								
Interval	0	1	2	3	4	5	6	7	8
3 - 3.99	1	0	0	0	0	0	0	0	0
5 - 5.99	0	0.77	0.23	0	0	0	0	0	0
6 - 6.99	0	0.46	0.54	0	0	0	0	0	0
7 - 7.99	0	0.29	0.64	0.07	0	0	0	0	0
8 - 8.99	0	0.09	0.64	0.27	0	0	0	0	0
9 - 9.99	0	0	0.86	0.14	0	0	0	0	0
10 - 10.99	0	0	0.47	0.47	0.07	0	0	0	0
11 - 11.99	0	0	0.35	0.65	0	0	0	0	0
12 - 12.99	0	0	0	0.4	0.6	0	0	0	0
13 - 13.99	0	0	0	0.37	0.63	0	0	0	0
14 - 14.99	0	0	0	0.33	0.58	0	0.08	0	0
15 - 15.99	0	0	0	0	0.79	0.14	0.07	0	0
16 - 16.99	0	0	0	0	0.71	0	0.29	0	0
17 - 17.99	0	0	0	0	0.25	0	0.5	0.25	0
18 - 18.99	0	0	0	0	0.11	0.22	0.67	0	0
19 - 19.99	0	0	0	0	0.2	0	0.2	0.6	0
20 - 20.99	0	0	0	0	0.2	0	0.4	0.2	0.2
21 - 21.99	0	0	0	0	0	0.5	0.5	0	0

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Chapter 8

SPANISH MACKEREL
Scomberomorous maculatus



8.1 INTRODUCTION

We aged a total of 210 Spanish Mackerel *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Spanish Mackerel ages ranged from 0 to 7 years old with an average age of 2.1, a standard deviation of 1.7, and a standard error of 0.12. Eight age classes (0 to 7) were represented, comprising fish of the 2015 to 2022 year-classes. The sample was dominated by fish from the year-class of 2021 with 41%.

8.2 METHODS

8.2.1 Sample Size for Ageing

We estimated sample size for ageing Spanish Mackerel in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Spanish Mackerel in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spanish Mackerel used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Spanish Mackerel collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

8.2.2 Handling of Collections

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

8.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otolith", were processed for age determination. The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

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

8.2.4 Readings

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

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

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

For example, Spanish Mackerel otolith annulus formation occurs between May and June (Schmidt et al. 1993). A Spanish Mackerel with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after June and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 3 ($2 + 1 = 3$). When it is captured between May and June, it is Age 2 when its margin code is "2" but Age 3 ($2 + 1 = 3$) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 8.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.



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

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

fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

8.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

8.3 RESULTS

8.3.1 Sample Size

We estimated a sample size of 228 Spanish Mackerel in 2022, ranging in length interval from 12 to 34 inches (Table 8.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 8.2). In 2022, we randomly selected and aged 210 fish from 295 Spanish Mackerel collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 31 fish. We were not short of any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

8.3.2 Year Class

Of the 210 fish aged with otoliths, 8 age classes (0 to 7) were represented (Table 8.3). The average age was 2.1 years, and the standard deviation and standard error were 1.7 and 0.12, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2015 to 2022 year-classes, with fish primarily from the year class of 2021 with 41%. The ratio of males to females was 1:2.27 in the sample collected (Figure 8.2).

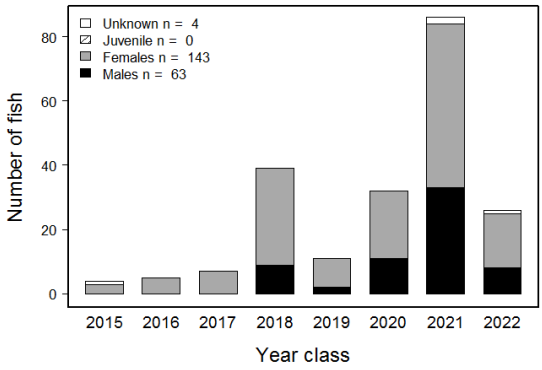


Figure 8.2: Year-class frequency distribution for Spanish Mackerel collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.3 Age-length Key (ALK)

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

8.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 96% and a CV of 1.26% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 0.72% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 97.62% and a CV of 0.78% (test of symmetry: $\chi^2 = 1$, $df = 3$, $P = 0.8013$) (Figure 8.3).

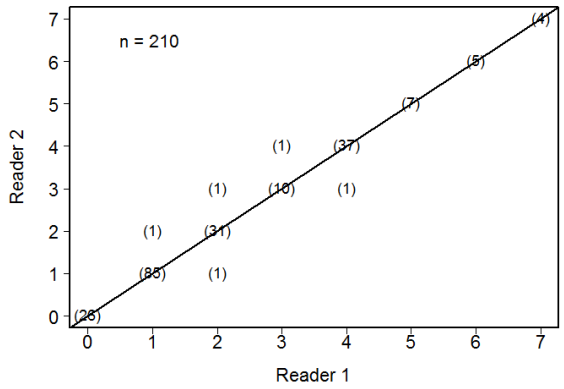


Figure 8.3: Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with fish aged in 2003 with a CV of 0.4% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100% .

Table 8.1: Number of Spanish Mackerel collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
12 - 12.99	5	0	0	5
13 - 13.99	5	0	0	5
14 - 14.99	16	19	16	0
15 - 15.99	28	32	28	0
16 - 16.99	31	37	32	0
17 - 17.99	27	46	28	0
18 - 18.99	16	22	16	0
19 - 19.99	14	18	14	0
20 - 20.99	10	19	10	0
21 - 21.99	10	16	10	0
22 - 22.99	6	14	6	0
23 - 23.99	5	14	6	0
24 - 24.99	5	10	6	0
25 - 25.99	5	8	6	0
26 - 26.99	5	10	6	0
27 - 27.99	5	10	6	0
28 - 28.99	5	10	10	0
29 - 29.99	5	6	6	0
30 - 30.99	5	1	1	4
31 - 31.99	5	1	1	4
32 - 32.99	5	0	0	5
33 - 33.99	5	1	1	4
34 - 34.99	5	1	1	4
Totals	228	295	210	31

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Table 8.2: CV for each age estimated based on ageing the total of 228 Spanish Mackerel in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spanish Mackerel collected from 2016 to 2020.

Age	CV	Percent
0	>0.25	1.01
1	0.05	49.91
2	0.09	31.43
3	0.17	10.85
4	>0.25	4.23
5	>0.25	1.38
6	>0.25	0.74
7	>0.25	0.46

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Table 8.3: The number of Spanish Mackerel assigned to each total length-at-age category for 210 fish sampled for otolith age determination in Virginia during 2022.

	Age								
Interval	0	1	2	3	4	5	6	7	Totals
14 - 14.99	15	1	0	0	0	0	0	0	16
15 - 15.99	11	17	0	0	0	0	0	0	28
16 - 16.99	0	30	2	0	0	0	0	0	32
17 - 17.99	0	24	4	0	0	0	0	0	28
18 - 18.99	0	8	5	1	2	0	0	0	16
19 - 19.99	0	3	10	0	1	0	0	0	14
20 - 20.99	0	2	4	1	3	0	0	0	10
21 - 21.99	0	1	3	4	2	0	0	0	10
22 - 22.99	0	0	3	0	3	0	0	0	6
23 - 23.99	0	0	1	3	2	0	0	0	6
24 - 24.99	0	0	0	1	5	0	0	0	6
25 - 25.99	0	0	0	0	5	0	1	0	6
26 - 26.99	0	0	0	0	6	0	0	0	6
27 - 27.99	0	0	0	1	3	2	0	0	6
28 - 28.99	0	0	0	0	6	2	2	0	10
29 - 29.99	0	0	0	0	1	3	1	1	6
30 - 30.99	0	0	0	0	0	0	0	1	1
31 - 31.99	0	0	0	0	0	0	0	1	1
33 - 33.99	0	0	0	0	0	0	1	0	1
34 - 34.99	0	0	0	0	0	0	0	1	1
Totals	26	86	32	11	39	7	5	4	210

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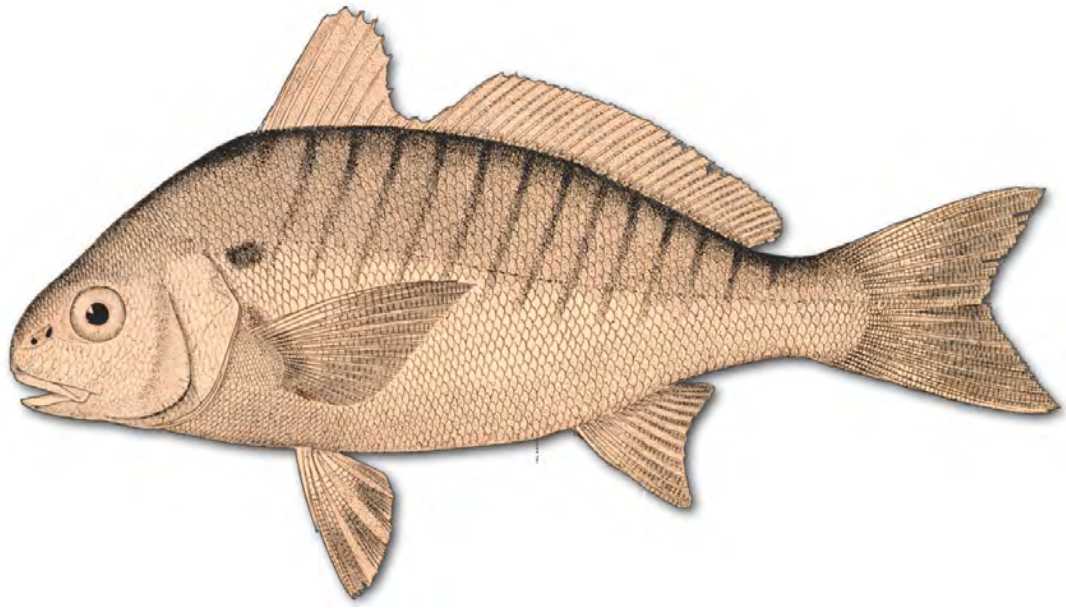
Table 8.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spanish Mackerel sampled for age determination in Virginia during 2022.

	Age							
Interval	0	1	2	3	4	5	6	7
14 - 14.99	0.94	0.06	0	0	0	0	0	0
15 - 15.99	0.39	0.61	0	0	0	0	0	0
16 - 16.99	0	0.94	0.06	0	0	0	0	0
17 - 17.99	0	0.86	0.14	0	0	0	0	0
18 - 18.99	0	0.5	0.31	0.06	0.12	0	0	0
19 - 19.99	0	0.21	0.71	0	0.07	0	0	0
20 - 20.99	0	0.2	0.4	0.1	0.3	0	0	0
21 - 21.99	0	0.1	0.3	0.4	0.2	0	0	0
22 - 22.99	0	0	0.5	0	0.5	0	0	0
23 - 23.99	0	0	0.17	0.5	0.33	0	0	0
24 - 24.99	0	0	0	0.17	0.83	0	0	0
25 - 25.99	0	0	0	0	0.83	0	0.17	0
26 - 26.99	0	0	0	0	1	0	0	0
27 - 27.99	0	0	0	0.17	0.5	0.33	0	0
28 - 28.99	0	0	0	0	0.6	0.2	0.2	0
29 - 29.99	0	0	0	0	0.17	0.5	0.17	0.17
30 - 30.99	0	0	0	0	0	0	0	1
31 - 31.99	0	0	0	0	0	0	0	1
33 - 33.99	0	0	0	0	0	0	1	0
34 - 34.99	0	0	0	0	0	0	0	1

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Chapter 9

SPOT *Leiostomus xanthurus*



9.1 INTRODUCTION

We aged a total of 172 Spot *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Spot ages ranged from 0 to 4 years old with an average age of 1.2, a standard deviation of 0.5, and a standard error of 0.04. Five age classes (0 to 4) were represented, comprising fish of the 2018 to 2022 year-classes. The sample was dominated by fish from the year-class of 2021 with 82.6%.

9.2 METHODS

9.2.1 Sample Size for Ageing

We estimated sample size for ageing Spot in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Spot in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spot used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Spot collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

9.2.2 Handling of Collections

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

9.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Barbieri et al. (1994) with a few modifications. The left or right otolith was randomly selected and embedded (distal side down) in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

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

9.2.4 Readings

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

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

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

For example, Spot otolith annulus formation occurs between May and July (Piner and Jones 2004). A Spot with one visible annuli could be assigned an age of 1 or 2 depending on its capture month and margin code. When its margin code is "1", it is Age 1 no matter when it is captured. When it is captured after July and before January, it is Age 1 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 2 (1 + 1 = 2). When it is captured between May and July, it is Age 1 when its margin code is "2" but Age 2 (1 + 1 = 2) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 9.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

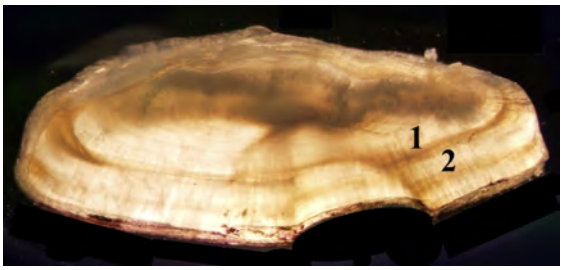


Figure 9.1: Otolith thin-section of a 2 year-old Spot

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

able to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

9.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

9.3 RESULTS

9.3.1 Sample Size

We estimated a sample size of 170 Spot in 2022, ranging in length interval from 4 to 12 inches (Table 9.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 4% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 9.2). In 2022, we randomly selected and aged 172 fish from 197 Spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 23 fish. We were short of only a few fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

9.3.2 Year Class

Of the 172 fish aged with otoliths, 5 age classes (0 to 4) were represented (Table 9.3). The average age was 1.2 years, and the standard deviation and standard error were 0.5 and 0.04, respectively. Year-class data show that the fishery was comprised of 5 year-classes: fish from the 2018 to 2022 year-classes, with fish primarily from the year class of 2021 with 82.6%. The ratio of males to females was 1:15 in the sample collected (Figure 9.2).

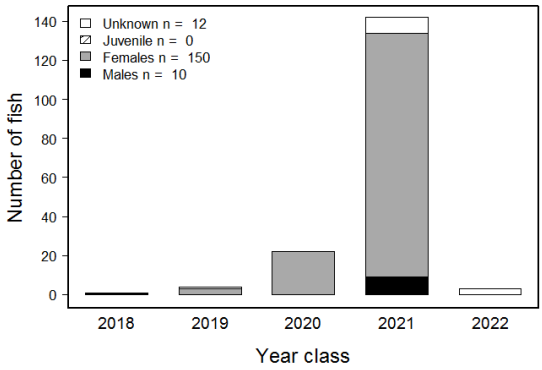


Figure 9.2: Year-class frequency distribution for Spot collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

9.3.3 Age-length Key (ALK)

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

9.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 96% and a CV of 2.36% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$),

and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 1.35% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 95.93% and a CV of 1.76% (test of symmetry: $\chi^2 = 1.67$, $df = 2$, $P = 0.4346$) (Figure 9.3).

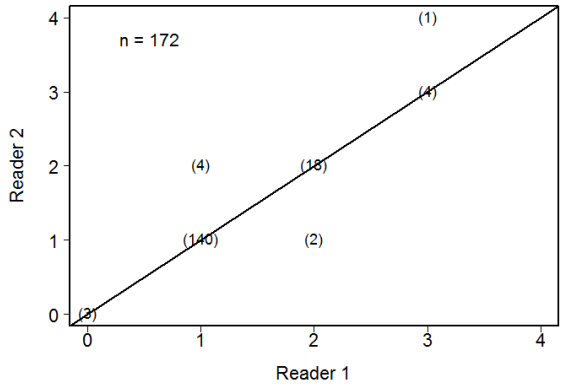


Figure 9.3: Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2000. Reader 2 had an agreement of 98% with a CV of 0.94% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

Table 9.1: Number of Spot collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	2	2	3
5 - 5.99	5	1	1	4
6 - 6.99	5	11	11	0
7 - 7.99	20	30	23	0
8 - 8.99	36	45	37	0
9 - 9.99	53	78	68	0
10 - 10.99	36	29	29	7
11 - 11.99	5	1	1	4
12 - 12.99	5	0	0	5
Totals	170	197	172	23

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Table 9.2: CV for each age estimated based on ageing the total of 170 Spot in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spot collected from 2016 to 2020.

Age	CV	Percent
0	0.18	4.84
1	0.04	76.55
2	0.17	15.88
3	>0.25	1.82
4	>0.25	0.64
5	>0.25	0.27

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Table 9.3: The number of Spot assigned to each total length-at-age category for 172 fish sampled for otolith age determination in Virginia during 2022.

	Age					
Interval	0	1	2	3	4	Totals
4 - 4.99	2	0	0	0	0	2
5 - 5.99	1	0	0	0	0	1
6 - 6.99	0	11	0	0	0	11
7 - 7.99	0	23	0	0	0	23
8 - 8.99	0	24	11	2	0	37
9 - 9.99	0	58	8	1	1	68
10 - 10.99	0	25	3	1	0	29
11 - 11.99	0	1	0	0	0	1
Totals	3	142	22	4	1	172

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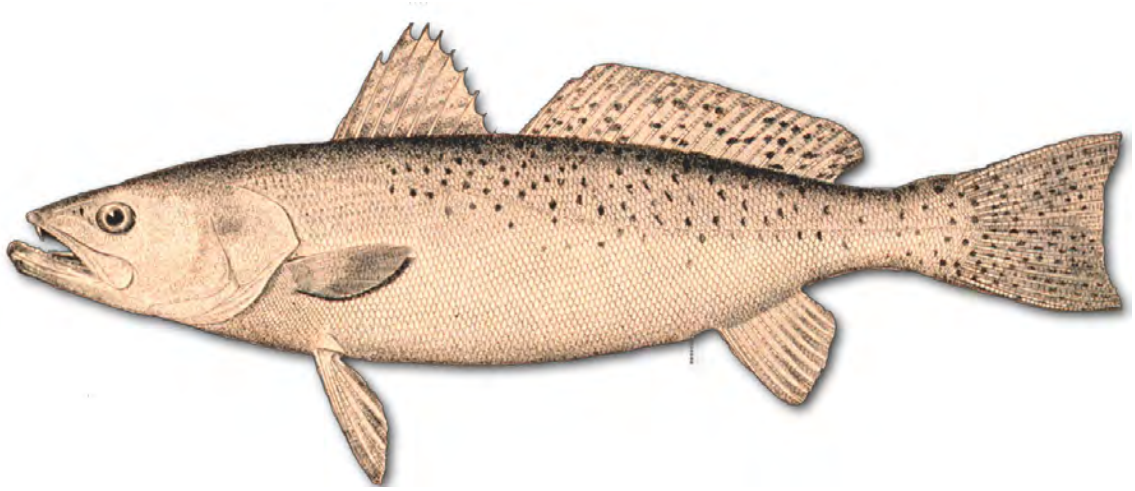
Table 9.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spot sampled for age determination in Virginia during 2022.

	Age				
Interval	0	1	2	3	4
4 - 4.99	1	0	0	0	0
5 - 5.99	1	0	0	0	0
6 - 6.99	0	1	0	0	0
7 - 7.99	0	1	0	0	0
8 - 8.99	0	0.65	0.3	0.05	0
9 - 9.99	0	0.85	0.12	0.01	0.01
10 - 10.99	0	0.86	0.1	0.03	0
11 - 11.99	0	1	0	0	0

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Chapter 10

SPOTTED SEATROUT *Cynoscion nebulosus*



10.1 INTRODUCTION

We aged a total of 283 Spotted Seatrout *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. Spotted seatrout ages ranged from 0 to 5 years old with an average age of 1.7, a standard deviation of 1.2, and a standard error of 0.07. Six age classes (0 to 5) were represented, comprising fish of the 2017 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2020 and 2021 with 26.1% and 36.8%, respectively.

10.2 METHODS

10.2.1 Sample Size for Ageing

We estimated sample size for ageing Spotted Seatrout in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Spotted Seatrout in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spotted Seatrout used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Spotted Seatrout collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

10.2.2 Handling of Collections

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

10.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

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

for ageing Spotted Seatrout.

10.2.4 Readings

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

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

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

For example, Spotted Seatrout otolith annulus formation occurs between March and May (Ihde and Chittenden 2003). A Spotted Seatrout with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after May and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before March and its margin code is not "1", it is Age 3 (2 + 1 = 3). When it is captured between March and May, it is Age 2 when its margin code is "2" but Age 3 (2 + 1 = 3) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 10.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

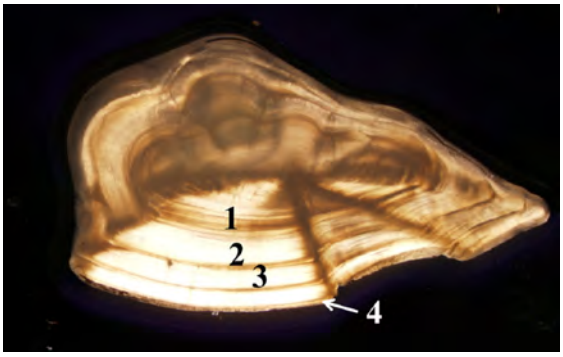


Figure 10.1: Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated

ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

10.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

10.3 RESULTS

10.3.1 Sample Size

We estimated a sample size of 299 Spotted Seatrout in 2022, ranging in length interval from 7 to 31 inches (Table 10.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 10.2). In 2022, we randomly selected and aged 283 fish from 549 Spotted Seatrout collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 32 fish. We were not short of any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

10.3.2 Year Class

Of the 283 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table 10.3). The average age was 1.7 years, and the standard deviation and standard error were 1.2 and 0.07, respectively. Year-class data show that the fishery was comprised of 6 year-classes: fish from the 2017 to 2022 year-classes, with fish primarily from the year classes of 2020 and 2021 with 26.1% and 36.8%, respectively. The ratio of males to females was 1:1.33 in the sample collected (Figure 10.2).

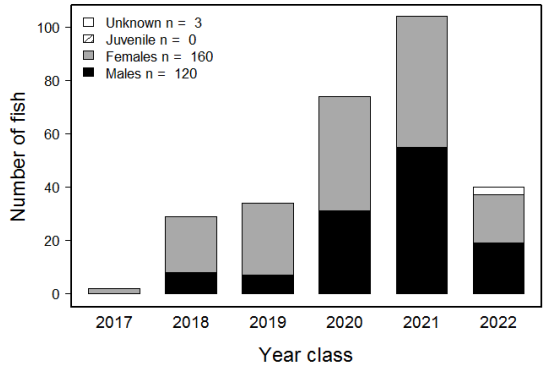


Figure 10.2: Year-class frequency distribution for Spotted Seatrout collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

10.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 10.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based

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

10.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 100%, and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 10.3).

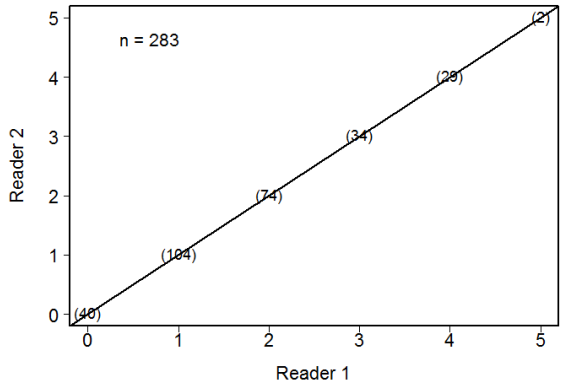


Figure 10.3: Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2000. Reader 2 also had an agreement of 100%.

Table 10.1: Number of Spotted Seatrout collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
7 - 7.99	5	0	0	5
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	5	9	9	0
11 - 11.99	8	41	8	0
12 - 12.99	17	64	18	0
13 - 13.99	14	18	14	0
14 - 14.99	15	26	16	0
15 - 15.99	22	70	22	0
16 - 16.99	29	67	30	0
17 - 17.99	29	56	30	0
18 - 18.99	24	39	24	0
19 - 19.99	21	32	22	0
20 - 20.99	20	19	19	1
21 - 21.99	12	15	12	0
22 - 22.99	13	31	14	0
23 - 23.99	9	15	10	0
24 - 24.99	9	17	10	0
25 - 25.99	7	11	8	0
26 - 26.99	5	8	6	0
27 - 27.99	5	7	7	0
28 - 28.99	5	2	2	3
29 - 29.99	5	1	1	4
30 - 30.99	5	0	0	5
31 - 31.99	5	1	1	4
Totals	299	549	283	32

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Table 10.2: CV for each age estimated based on ageing the total of 299 Spotted Seatrout in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Spotted Seatrout collected from 2016 to 2020.

Age	CV	Percent
0	0.14	8.78
1	0.05	46.89
2	0.07	33.53
3	0.15	8.93
4	>0.25	1.65
5	>0.25	0.23

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Table 10.3: The number of Spotted Seatrout assigned to each total length-at-age category for 283 fish sampled for otolith age determination in Virginia during 2022.

	Age						
Interval	0	1	2	3	4	5	Totals
10 - 10.99	9	0	0	0	0	0	9
11 - 11.99	8	0	0	0	0	0	8
12 - 12.99	18	0	0	0	0	0	18
13 - 13.99	5	9	0	0	0	0	14
14 - 14.99	0	16	0	0	0	0	16
15 - 15.99	0	21	1	0	0	0	22
16 - 16.99	0	27	3	0	0	0	30
17 - 17.99	0	14	14	2	0	0	30
18 - 18.99	0	9	15	0	0	0	24
19 - 19.99	0	4	14	3	1	0	22
20 - 20.99	0	4	12	2	1	0	19
21 - 21.99	0	0	6	4	2	0	12
22 - 22.99	0	0	5	7	2	0	14
23 - 23.99	0	0	3	5	2	0	10
24 - 24.99	0	0	0	7	2	1	10
25 - 25.99	0	0	1	2	5	0	8
26 - 26.99	0	0	0	2	4	0	6
27 - 27.99	0	0	0	0	7	0	7
28 - 28.99	0	0	0	0	2	0	2
29 - 29.99	0	0	0	0	1	0	1
31 - 31.99	0	0	0	0	0	1	1
Totals	40	104	74	34	29	2	283

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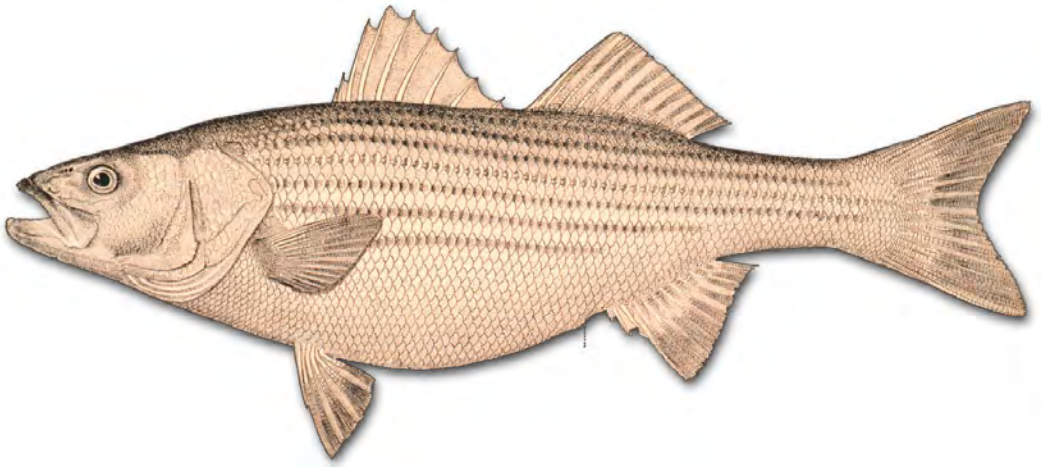
Table 10.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spotted Seatrout sampled for age determination in Virginia during 2022.

	Age					
Interval	0	1	2	3	4	5
10 - 10.99	1	0	0	0	0	0
11 - 11.99	1	0	0	0	0	0
12 - 12.99	1	0	0	0	0	0
13 - 13.99	0.36	0.64	0	0	0	0
14 - 14.99	0	1	0	0	0	0
15 - 15.99	0	0.95	0.05	0	0	0
16 - 16.99	0	0.9	0.1	0	0	0
17 - 17.99	0	0.47	0.47	0.07	0	0
18 - 18.99	0	0.38	0.62	0	0	0
19 - 19.99	0	0.18	0.64	0.14	0.05	0
20 - 20.99	0	0.21	0.63	0.11	0.05	0
21 - 21.99	0	0	0.5	0.33	0.17	0
22 - 22.99	0	0	0.36	0.5	0.14	0
23 - 23.99	0	0	0.3	0.5	0.2	0
24 - 24.99	0	0	0	0.7	0.2	0.1
25 - 25.99	0	0	0.12	0.25	0.62	0
26 - 26.99	0	0	0	0.33	0.67	0
27 - 27.99	0	0	0	0	1	0
28 - 28.99	0	0	0	0	1	0
29 - 29.99	0	0	0	0	1	0
31 - 31.99	0	0	0	0	0	1

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Chapter 11

STRIPED BASS *Morone saxatilis*



11.1 INTRODUCTION

We aged a total of 820 Striped Bass *Morone saxatilis*, collected by the VMRC's Biological Sampling Program in 2022. Of 820 aged fish, 527 and 293 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 8.4 years with a standard deviation of 5.3 and a standard error of 0.23. Twenty-five age classes (2 to 24, 26, and 29) were represented in the bay fish, comprising fish from the 1993, 1996, and 1998 to 2020 year classes. The bay fish sample in 2022 was dominated by the year classes of 2011, 2014, 2015, 2016, 2017, and 2018 with 8%, 9%, 21%, 8%, 9%, 18%, and 5%, respectively. The average ocean fish age was 11.8 years with a standard deviation of 3.3 and a standard error of 0.19. Twenty age classes (7 to 24, and 26 to 27) were represented in the ocean fish, comprising fish from the 1995 to 1996, and 1998 to 2015 year classes. The ocean fish sample in 2022 was dominated by the year classes of 2010, 2011, 2012, 2013, and 2014 with 15%, 35%, 12%, 8%, and 7%, respectively. We also aged 305 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

11.2 METHODS

11.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Striped Bass collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Striped Bass in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Striped Bass used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Striped Bass collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

11.2.2 Handling of Collection

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

11.2.3 Preparation

11.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly

selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

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

11.2.3.2 *Scales*

Striped bass scales were prepared for age and growth analysis by making acetate impressions of the scale microstructure. Due to extreme variation in the size and shape of scales from individual fish, we selected only those scales that had even margins and which were of uniform size. We selected a range of four to six preferred scales (based on overall scale size) from each fish, making sure that only non-regenerated scales were used. Scale impressions were made on extruded clear acetate sheets (25 mm x 75 mm) with a Carver Laboratory Heated Press (model "C"). The scales were pressed with the following settings:

Pressure: 15000 psi
Temperature: 77 °C (170 °F)
Time: 5 to 10 min

Striped bass scales that were the size of a quarter (coin) or larger, were pressed individually for up to twenty minutes. After pressing, the impressions were viewed with a Bell and Howell microfiche reader and checked again for regeneration and incomplete margins. Impressions that were too light, or when all scales were regenerated a new impression was made using different scales from the same fish.

Click here to obtain the protocol at the VMRC website on how to prepare scale impression for ageing Striped Bass.

11.2.4 Readings

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

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

preted as either being toward the next age class or within the same age class. If translucent growth is visible beyond the last dark annulus, a "+" is added to the notation.

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

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

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

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

11.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 11.1). 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 section. The focus is generally located, depending on preparation, in the center of the otolith section, 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 section. This insertion point along the sulcal ridge resembles a check mark (not to be confused with a false annulus). Here the annulus can be followed outwards along the ventral and dorsal surfaces where it encircles the focus. Subsequent annuli also emanate from the sulcal ridge; however, they do not encircle the focus, but rather travel outwards to the distal surface of the otolith. To be considered a true annulus, each annulus must be rooted in the sulcus and travel without interruption to the distal surface of the otolith. The annuli in Striped Bass have a tendency to split as they advance towards the distal surface. As a result, it is critical that reading path proceed in a direction down the sulcal ridge and outwards to the distal surface.

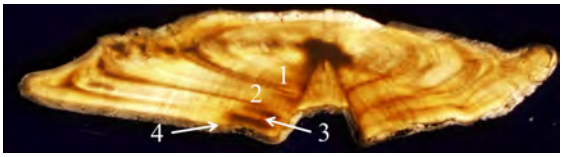


Figure 11.1: Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section

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

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

11.2.4.2 *Scales*

We determined fish age by viewing acetate impressions of scales (Figure 11.2) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Annuli on

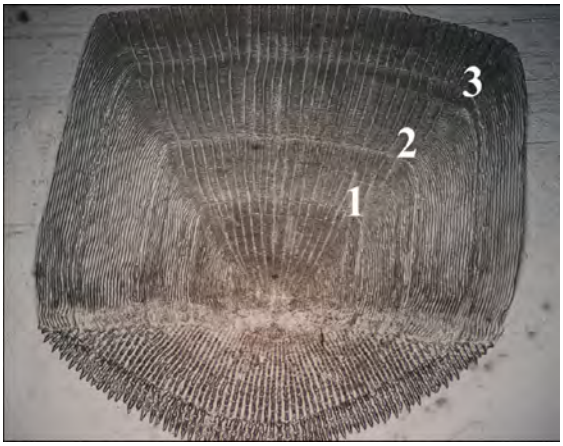


Figure 11.2: Scale impression of a 3 year-old Striped Bass.

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

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

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

11.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used

to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

11.3 RESULTS

11.3.1 Sample Size

The sample sizes are estimated for Striped Bass in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lengths, with both otoliths and scales, otolith-only, and scale-only. The total sample aged from each area may be smaller than or equal to the total sample size.

11.3.1.1 *Chesapeake Bay*

We estimated a sample size of 530 bay Striped Bass in 2022, ranging in length interval from 10 to 55 inches (Table 11.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 5 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 11.2). We aged all the fish with both scales and otoliths (241 fish). We aged 1 fish with otolith-only. We randomly selected 285 fish with scale-only to age. As a result, we aged 527 of 756 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2022. We fell short in our over-all collections for the optimal length-class sampling estimate by 80 fish. We were short only a few fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

11.3.1.2 *Atlantic Ocean*

We estimated a sample size of 501 ocean Striped Bass in 2022, ranging in length interval from 28 to 53 inches (Table 11.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 11% for the major age of Age 10 and 11 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 11.4). We aged all the fish with both scales and otoliths (64 fish). We randomly selected 229 fish with scale-only to age. As a result, we aged 293 of 313 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Virginia waters of the Atlantic Ocean in 2022. We fell short in our over-all collections for the optimal length-class sampling estimate by 210 fish. We were short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

11.3.2 Year Class

The year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The otolith ages are always the primary ages. When otolith ages are not available, the scale ages are used.

11.3.2.1 *Chesapeake Bay*

Of the 527 bay Striped Bass aged, 25 age classes (2 to 24, 26, and 29) were represented (Table 11.5). The average age for the sample was 8.4 years. The standard deviation and standard error were 5.3 and 0.23, respectively. Year-class data (Figure 11.3) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2020 year-class for Striped Bass caught in 2022. Striped bass in the sample in 2022 was dominated by the year classes of 2011, 2014, 2015, 2016, 2017, and 2018 with 8%, 9%, 21%, 8%, 9%, 18%, and 5%, respectively. The sex ratio of male to female was 1:1.21 for the bay fish.

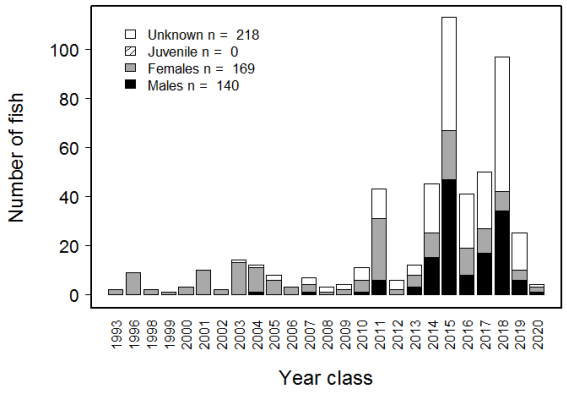


Figure 11.3: Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.2.2 *Atlantic Ocean*

Of the 293 ocean Striped Bass aged, 20 age classes (7 to 24, and 26 to 27) were represented (Table 11.6). The average age for the sample was 11.8 years. The standard deviation and standard error were 3.3 and 0.19, respectively. Year-class data (Figure 11.4) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 7, which corresponds to the 2015 year-class for Striped Bass caught in 2022. Striped bass in the sample in 2022 was dominated by the year classes of 2010, 2011, 2012, 2013, and 2014 with 15%, 35%, 12%, 8%, and 7%, respectively. The sex ratio of male to female was 1:6.86 for the ocean fish.

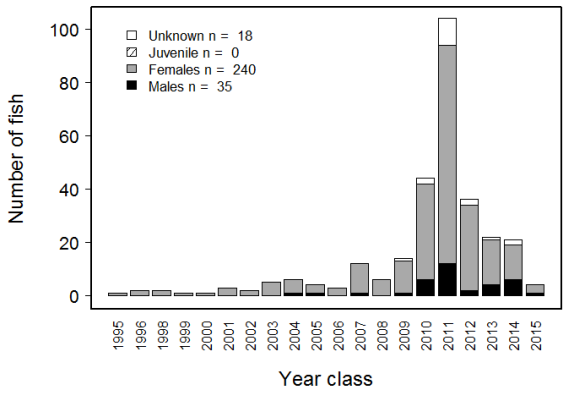


Figure 11.4: Year-class frequency distribution for Striped Bass collected in Virginia waters of the Atlantic Ocean for ageing in 2022. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.3 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 11.7) and ocean fish (Table 11.8) using scale ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

11.3.4 Reading Precision

11.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 306 Striped Bass collected in 2022. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 84% and a CV of 0.8% (test of symmetry: $\chi^2 = 8$, $df = 7$, $P = 0.3326$), and there was

no significant difference between the first and second readings for Reader 2 with an agreement of 90% and a CV of 0.3% (test of symmetry: $\chi^2 = 5$, $df = 5$, $P = 0.4159$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 92% (1 year or less agreement of 99%) and a CV of 0.4% (test of symmetry: $\chi^2 = 17$, $df = 16$, $P = 0.3856$) (Figure 11.5).

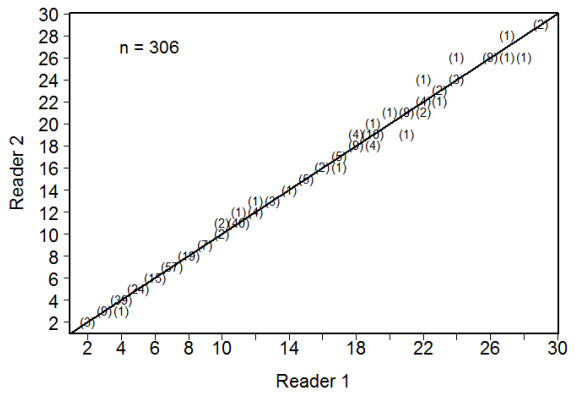


Figure 11.5: Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 83% with ages of fish aged in 2000 with a CV of 1.8% (test of symmetry: $\chi^2 = 10$, $df = 7$, $P = 0.1886$). Reader 2 had an agreement of 88% with a CV of 1.1% (test of symmetry: $\chi^2 = 7$, $df = 6$, $P = 0.3208$).

11.3.4.2 *Scales*

Reader 1 and Reader 2 the scales of 819 Striped Bass collected in 2022. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 76% (1 year or less agreement of 98%) and a CV of 1.8% (test of symmetry: $\chi^2 = 9.3$, $df = 9$, $P = 0.4071$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 62% (1 year or less agreement of 94%) and a CV of 3.9% (test of symmetry: $\chi^2 = 17$, $df = 14$, $P = 0.2562$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 64% (1 year or less agreement of 91%) and a CV of 3.8% (test of symmetry: $\chi^2 = 102.2$, $df = 51$, $P < 0.0001$) (Figure 11.6).

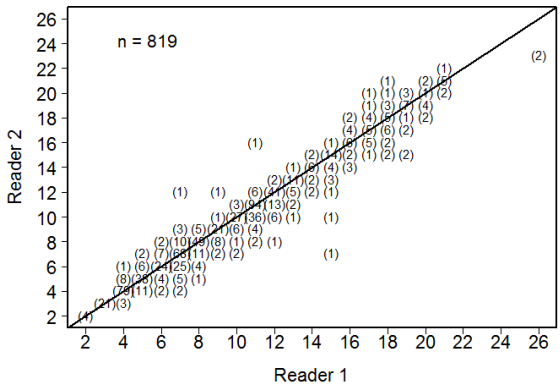


Figure 11.6: Between-reader comparison of scale age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 60% (1 year or less agreement of 93%) with ages of fish aged in 2000 with a CV of 4.9% (test of symmetry: $\chi^2 = 11.3$, $df = 14$, $P = 0.6597$). Reader 2 had an agreement of 63% (1 year or less agreement of 97%) with a CV of 4.7% (test of symmetry: $\chi^2 = 14.7$, $df = 12$, $P = 0.2602$).

11.3.5 Comparison of Scale and Otolith Ages

We aged 305 pairs of Striped Bass scales and otoliths. There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 102.8$, $df = 48$, $P < 0.0001$) with an average CV of 5%. There was an agreement of 57% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 37% and 6.2% of the fish, respectively (Figure 11.7). There was also an evidence of bias between otolith and scale ages using an age bias plot (Figure 11.8),

with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

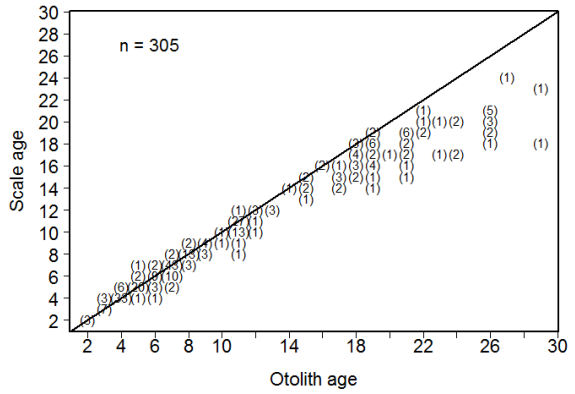


Figure 11.7: Comparison of scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

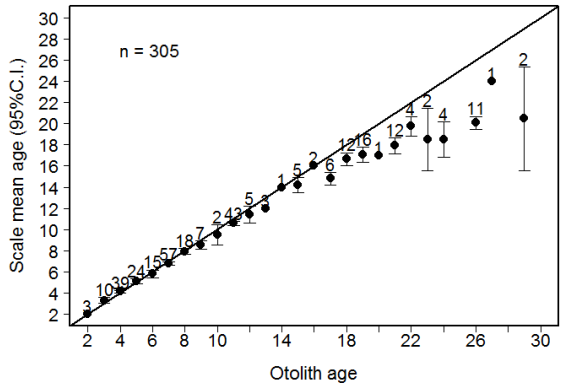


Figure 11.8: Age-bias plot for Striped Bass scale and otolith age estimates in 2022. The number above the upper CI bar is number of fish.

11.4 RECOMMENDATIONS

We recommend that VMRC and ASMFC use otoliths for ageing Striped Bass. Although preparation time is greater for otoliths compared to scales, nonetheless as the mean age of Striped Bass increases in the recovering fishery, otoliths should provide more reliable estimates of age (Secor et al. 1995; Liao et al. 2013). We will continue to compare the age estimates between otoliths and scales.

Table 11.1: Number of bay Striped Bass collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
10 - 10.99	5	0	0	5
11 - 11.99	5	0	0	5
12 - 12.99	5	0	0	5
13 - 13.99	5	0	0	5
14 - 14.99	5	0	0	5
15 - 15.99	5	0	0	5
16 - 16.99	5	0	0	5
17 - 17.99	5	0	0	5
18 - 18.99	12	30	21	0
19 - 19.99	26	47	33	0
20 - 20.99	32	52	33	0
21 - 21.99	27	53	30	0
22 - 22.99	25	51	29	0
23 - 23.99	26	51	27	0
24 - 24.99	24	42	25	0
25 - 25.99	21	34	23	0
26 - 26.99	21	37	23	0
27 - 27.99	18	41	24	0
28 - 28.99	16	25	17	0
29 - 29.99	14	21	14	0
30 - 30.99	13	23	14	0
31 - 31.99	13	17	14	0
32 - 32.99	16	19	17	0
33 - 33.99	14	20	15	0
34 - 34.99	13	14	14	0
35 - 35.99	14	14	14	0
36 - 36.99	14	9	9	5
37 - 37.99	16	7	7	9
38 - 38.99	14	8	8	6
39 - 39.99	10	15	10	0
40 - 40.99	8	16	8	0
41 - 41.99	8	17	17	0
42 - 42.99	8	10	8	0
43 - 43.99	8	12	11	0
44 - 44.99	8	14	12	0
45 - 45.99	8	18	12	0
46 - 46.99	8	13	12	0
47 - 47.99	5	9	9	0
48 - 48.99	5	9	9	0
49 - 49.99	5	8	8	0
50 - 50.99	5	0	0	5
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
55 - 55.99	5	0	0	5
Totals	530	756	527	80

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Table 11.2: CV for each age estimated based on ageing the total of 530 bay Striped Bass in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Striped Bass collected from 2016 to 2020.

Age	CV	Percent
1	0.22	0.54
2	>0.25	0.51
3	0.23	3.3
4	0.11	11.99
5	0.09	17.67
6	0.11	12.57
7	0.14	8.56
8	0.14	7.92
9	0.14	8.09
10	0.19	4.86
11	0.19	4.55
12	0.21	3.98
13	0.23	3.19
14	>0.25	2.72
15	>0.25	1.94
16	>0.25	2.14
17	>0.25	1.22
18	>0.25	1.43
19	>0.25	1.02
20	>0.25	0.68
21	>0.25	0.58
22	>0.25	0.34
23	>0.25	0.17
24	>0.25	0.03

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Table 11.3: Number of ocean Striped Bass collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
28 - 28.99	5	0	0	5
29 - 29.99	5	1	1	4
30 - 30.99	5	0	0	5
31 - 31.99	5	2	2	3
32 - 32.99	9	1	1	8
33 - 33.99	15	1	1	14
34 - 34.99	23	4	4	19
35 - 35.99	40	15	15	25
36 - 36.99	56	22	22	34
37 - 37.99	65	31	31	34
38 - 38.99	59	39	39	20
39 - 39.99	40	45	40	0
40 - 40.99	39	51	40	0
41 - 41.99	35	39	36	0
42 - 42.99	22	21	21	1
43 - 43.99	14	10	9	5
44 - 44.99	12	9	9	3
45 - 45.99	7	5	5	2
46 - 46.99	10	5	5	5
47 - 47.99	9	4	4	5
48 - 48.99	6	3	3	3
49 - 49.99	5	3	3	2
50 - 50.99	5	1	1	4
51 - 51.99	5	1	1	4
53 - 53.99	5	0	0	5
Totals	501	313	293	210

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Table 11.4: CV for each age estimated based on ageing the total of 501 ocean Striped Bass in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Striped Bass collected from 2016 to 2020.

Age	CV	Percent
5	>0.25	0.08
6	>0.25	0.16
7	>0.25	1.95
8	0.13	9.83
9	0.12	12.79
10	0.11	13.57
11	0.11	13.96
12	0.13	11.31
13	0.14	9.67
14	0.15	8.42
15	0.18	5.62
16	0.22	3.74
17	0.24	3.28
18	>0.25	1.79
19	>0.25	1.56
20	>0.25	0.94
21	>0.25	0.62
22	>0.25	0.31
23	>0.25	0.23
25	>0.25	0.08

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Table 11.5: The number of Striped Bass assigned to each total length-at-age category for 527 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2022.

	Age																									
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	29	Totals
18 - 18.99	2	7	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
19 - 19.99	2	10	18	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
20 - 20.99	0	4	22	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
21 - 21.99	0	3	18	4	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30
22 - 22.99	0	1	10	9	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
23 - 23.99	0	0	6	6	3	10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27
24 - 24.99	0	0	3	5	4	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
25 - 25.99	0	0	1	7	4	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
26 - 26.99	0	0	0	5	3	11	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
27 - 27.99	0	0	0	1	2	14	5	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
28 - 28.99	0	0	3	0	4	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
29 - 29.99	0	0	3	2	1	3	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
30 - 30.99	0	0	0	2	0	4	4	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
31 - 31.99	0	0	0	1	3	5	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
32 - 32.99	0	0	1	1	4	6	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
33 - 33.99	0	0	0	1	2	7	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
34 - 34.99	0	0	0	1	1	6	4	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
35 - 35.99	0	0	0	0	0	5	8	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
36 - 36.99	0	0	0	0	0	3	2	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
37 - 37.99	0	0	0	0	0	0	0	1	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
38 - 38.99	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
39 - 39.99	0	0	0	0	0	0	1	0	0	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
40 - 40.99	0	0	0	0	0	0	0	2	1	2	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	8
41 - 41.99	0	0	0	0	0	0	0	0	1	9	3	1	0	0	1	0	1	1	0	0	0	0	0	0	0	17
42 - 42.99	0	0	0	0	0	0	0	0	0	1	2	1	0	1	0	2	0	1	0	0	0	0	0	0	0	8
43 - 43.99	0	0	0	0	0	0	0	0	0	2	0	2	0	2	0	1	1	1	0	1	0	0	1	0	0	11
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2	2	2	0	2	0	0	0	1	1	12
45 - 45.99	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	2	3	0	1	1	0	0	1	0	12
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	3	1	0	0	1	0	1	0	12
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	2	0	1	0	0	1	1	1	9
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	2	1	0	0	3	0	9
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	3	1	0	0	2	0	8
Totals	4	25	97	50	41	113	45	12	6	43	11	4	3	7	3	8	12	14	2	10	3	1	2	9	2	527

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Table 11.6: The number of Striped Bass assigned to each total length-at-age category for 293 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2022.

	Age																				
Interval	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	27	Totals
29 - 29.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
31 - 31.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
32 - 32.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
33 - 33.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
34 - 34.99	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
35 - 35.99	1	5	3	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
36 - 36.99	1	4	2	3	9	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	22
37 - 37.99	0	6	6	8	6	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	31
38 - 38.99	0	1	4	8	16	8	1	0	1	0	0	0	0	0	0	0	0	0	0	0	39
39 - 39.99	0	1	2	7	24	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	40
40 - 40.99	0	0	2	4	20	9	2	1	2	0	0	0	0	0	0	0	0	0	0	0	40
41 - 41.99	0	0	1	2	17	8	6	1	1	0	0	0	0	0	0	0	0	0	0	0	36
42 - 42.99	0	0	0	1	6	6	2	2	4	0	0	0	0	0	0	0	0	0	0	0	21
43 - 43.99	0	0	0	0	2	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	9
44 - 44.99	0	0	0	1	0	1	1	0	3	1	0	1	1	0	0	0	0	0	0	0	9
45 - 45.99	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0	1	0	0	0	0	5
46 - 46.99	0	0	0	0	0	0	0	0	1	0	0	2	1	0	0	0	1	0	0	0	5
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	1	4
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	3
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	3
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
51 - 51.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	4	21	22	36	104	44	14	6	12	3	4	6	5	2	3	1	1	2	2	1	293

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Table 11.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Chesapeake Bay, Virginia during 2022.

	Age																								
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	29
18 - 18.99	0.1	0.33	0.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0.06	0.3	0.55	0.06	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.12	0.67	0.09	0.09	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.1	0.6	0.13	0.1	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.03	0.34	0.31	0.14	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0.22	0.22	0.11	0.37	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0.12	0.2	0.16	0.44	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0.04	0.3	0.17	0.43	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0	0.22	0.13	0.48	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.04	0.08	0.58	0.21	0	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0.18	0	0.24	0.53	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0.21	0.14	0.07	0.21	0.21	0	0	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.14	0	0.29	0.29	0.07	0	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0.07	0.21	0.36	0.14	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0.06	0.06	0.24	0.35	0.24	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0.07	0.13	0.47	0.13	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0.07	0.07	0.43	0.29	0.07	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0.36	0.57	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0.33	0.22	0.33	0	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	0.14	0.43	0.29	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0.1	0	0	0.8	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0.25	0.12	0.25	0.12	0	0.12	0	0	0.12	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0.06	0.53	0.18	0.06	0	0	0.06	0	0.06	0.06	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0.12	0.25	0.12	0	0.12	0	0.25	0	0.12	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0.18	0	0.18	0	0.18	0	0.09	0.09	0.09	0	0.09	0	0	0.09	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0.08	0	0.08	0	0	0.17	0.17	0.17	0	0.17	0	0	0	0.08	0.08
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0.08	0	0.08	0.17	0	0	0.17	0.25	0	0.08	0.08	0	0	0.08	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.08	0.17	0.25	0.25	0.08	0	0	0.08	0	0.08	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0	0	0.22	0.22	0	0.11	0	0	0.11	0.11	0.11
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0	0.11	0	0.11	0.22	0.11	0	0	0.33	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0	0	0	0.12	0	0.38	0.12	0	0	0.25	0

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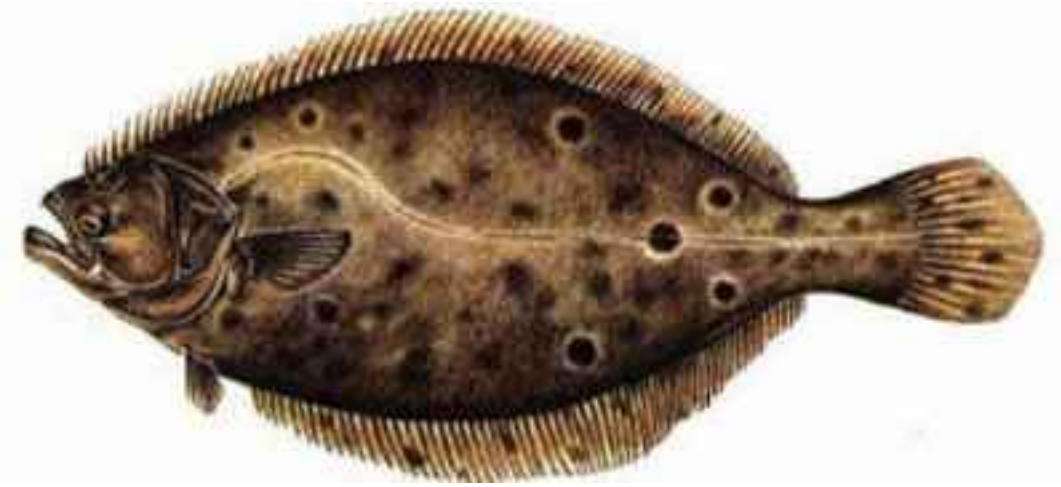
Table 11.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Virginia waters of the Atlantic Ocean during 2022.

	Age																			
Interval	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	27
29 - 29.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0.5	0.25	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0.07	0.33	0.2	0.13	0.2	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0.05	0.18	0.09	0.14	0.41	0.09	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0.19	0.19	0.26	0.19	0.13	0	0	0	0	0	0.03	0	0	0	0	0	0	0	0
38 - 38.99	0	0.03	0.1	0.21	0.41	0.21	0.03	0	0.03	0	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0.03	0.05	0.17	0.6	0.1	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0.05	0.1	0.5	0.22	0.05	0.03	0.05	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0.03	0.06	0.47	0.22	0.17	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0.05	0.29	0.29	0.1	0.1	0.19	0	0	0	0	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0.22	0.11	0.11	0.11	0	0.11	0.11	0.11	0	0.11	0	0	0	0	0	0
44 - 44.99	0	0	0	0.11	0	0.11	0.11	0	0.33	0.11	0	0.11	0.11	0	0	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0.2	0.4	0	0.2	0	0	0.2	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0.2	0	0	0.4	0.2	0	0	0	0.2	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0.5	0	0	0	0	0.25
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0.33	0.33	0	0	0	0	0.33	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0.33	0	0	0.33	0	0
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
51 - 51.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

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Chapter 12

SUMMER FLOUNDER *Paralichthys dentatus*



12.1 INTRODUCTION

We aged a total of 845 Summer Flounder *Paralichthys dentatus*, collected by the VMRC's Biological Sampling Program in 2022. Of 845 aged fish, 375 and 470 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 2.7 years with a standard deviation of 1.1 and a standard error of 0.06. Eight age classes (1 to 8) were represented in the bay fish, comprising fish from the 2014 to 2021 year classes. The bay fish sample in 2022 was dominated by the year classes of 2019 and 2020 with 24% and 51%, respectively. The average ocean fish age was 5.3 years with a standard deviation of 2.4 and a standard error of 0.11. Fourteen age classes (1 to 14) were represented in the ocean fish, comprising fish from the 2008 to 2021 year classes. The ocean fish sample in 2022 was dominated by the year classes of 2016, 2017, 2018, and 2019 with 14%, 19%, 19%, and 16%, respectively. We also aged 419 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

12.2 METHODS

12.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Summer Flounder collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Summer Flounder in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Summer Flounder used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Summer Flounder collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

12.2.2 Handling of Collection

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

12.2.3 Preparation

12.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors"

spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

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

12.2.3.2 *Scales*

Summer flounder scales were prepared for age and growth analysis by making acetate impressions of the scale microstructure. Due to extreme variation in the size and shape of scales from individual fish, we selected only those scales that had even margins and which were of uniform size. We selected a range of four to six preferred scales (based on overall scale size) from each fish, making sure that only non-regenerated scales were used. Scale impressions were made on extruded clear acetate sheets (25 mm x 75 mm) with a Carver Laboratory Heated Press (model "C"). The scales were pressed with the following settings:

Pressure: 15000 psi
Temperature: 77 °C (170 °F)
Time: 5 to 10 min

Summer Flounder scales that were the size of a quarter (coin) or larger, were pressed individually for up to twenty minutes. After pressing, the impressions were viewed with a Bell and Howell microfiche reader and checked again for regeneration and incomplete margins. Impressions that were too light, or when all scales were regenerated a new impression was made using different scales from the same fish.

Click here to obtain the protocol at the VMRC website on how to prepare scale impression for ageing Summer Flounder.

12.2.4 Readings

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

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

growth is visible beyond the last dark annulus, a "+" is added to the notation.

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

For example, Summer Flounder otolith deposition occurs between January and April (Bolz 1999). A Summer Flounder captured between January 1 and April 30, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of June 30, the period of annulus formation, which would be noted as 4+4.

Summer flounder scales are also considered to have a deposition between January and June (Bolz 1999 and modified by CQFE), and age class assignment using these hard-parts is conducted in the same way as otoliths.

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

12.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 12.1). 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 section. The focus is generally located, depending on preparation, in the center of the otolith, and is visually well defined as a dark oblong region. The first year's annulus can be located directly below the focus, along the outer ridge of the sulcal groove on the ventral and dorsal sides of the otolith. This insertion point along the sulcal ridge resembles a check mark (not to be confused with a false annulus). Here the annulus can be followed outwards along the ventral and dorsal surfaces where it encircles the focus. Subsequent annuli also emanate from the sulcal ridge; however, they do not encircle the focus, but rather travel outwards to the distal surface of the otolith. To be considered a true annulus, each annulus must be rooted in the sulcus and travel without interruption to the distal surface of the otolith. The annuli in Summer Flounder have a tendency to split as they advance towards the distal surface. As a result, it is critical that reading path proceed in a direction down the sulcal ridge and outwards to the distal surface.

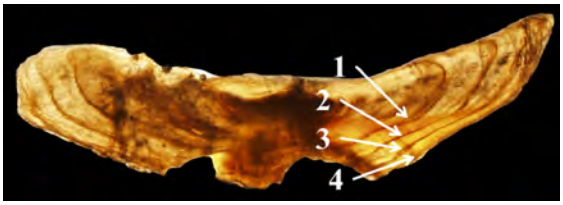


Figure 12.1: Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section

All thin-sections were aged by two different readers using a Nikon SMZ1000 stereo micro-

scope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples.

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

12.2.4.2 *Scales*

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

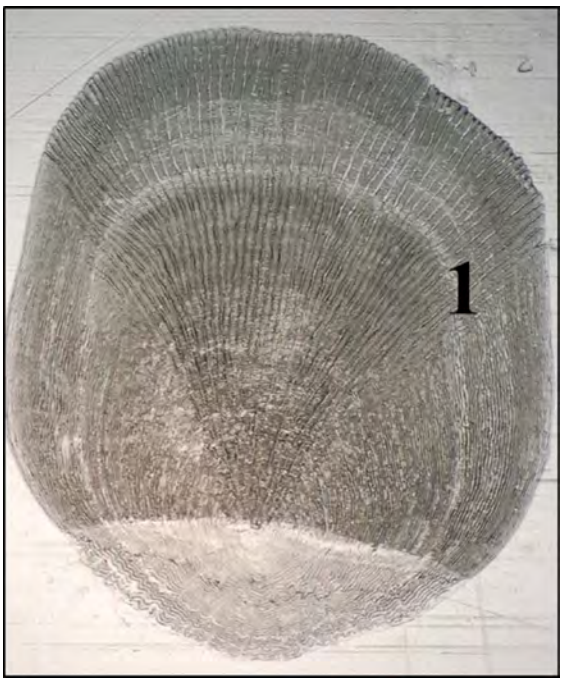


Figure 12.2: Scale impression of a 1 year-old Summer Flounder

on Summer Flounder scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

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

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

12.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

12.3 RESULTS

12.3.1 Sample Size

The sample sizes are estimated for Summer Flounder in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lengths, with both otoliths and scales, otolith-only, and scale-only. The total sample aged from each area may be smaller than or equal to the total sample size.

12.3.1.1 *Chesapeake Bay*

We estimated a sample size of 381 bay Summer Flounder in 2022, ranging in length interval from 8 to 30 inches (Table 12.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for the major age of Age 2 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 12.2). We aged all the fish with both scales and otoliths (112 fish). We aged 6 fish with otolith-only.We randomly selected 257 fish with scale-only to age. As a result, we aged 375 of 441 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2022. We fell short in our over-all collections for the optimal length-class sampling estimate by 46 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

12.3.1.2 *Atlantic Ocean*

We estimated a sample size of 478 ocean Summer Flounder in 2022, ranging in length interval from 13 to 32 inches (Table 12.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 4 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 12.4). We aged all the fish with both scales and otoliths (307 fish). We randomly selected 163 fish with scale-only to age. As a result, we aged 470 of 531 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2022. We fell short in our over-all collections for the optimal length-class sampling estimate by 36 fish. We were short some fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would possibly be influenced significantly.

12.3.2 Year class

The year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. When otolith ages are not available, the scale ages are used.

12.3.2.1 *Chesapeake Bay*

Of the 375 bay Summer Flounder aged, 8 age classes (1 to 8) were represented (Table

12.5). The average age for the sample was 2.7 years. The standard deviation and standard error were 1.1 and 0.06, respectively. Year-class data (Figure 12.3) indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2021 year-class for Summer Flounder caught in 2022. Summer flounder in the sample in 2022 was dominated by the year classes of 2019 and 2020 with 24% and 51%, respectively. The sex ratio of male to female was 1:116 for the bay fish.

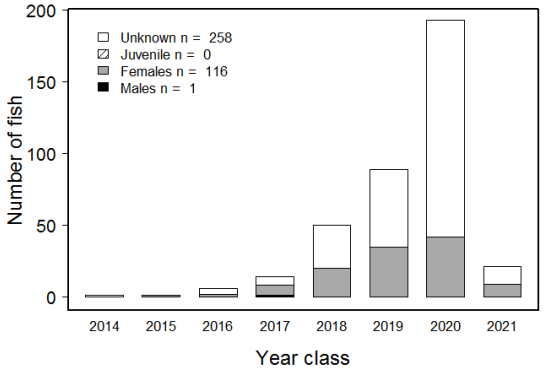


Figure 12.3: Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

12.3.2.2 *Atlantic Ocean*

Of the 470 ocean Summer Flounder aged, 14 age classes (1 to 14) were represented (Table 12.6). The average age for the sample was 5.3 years. The standard deviation and standard error were 2.4 and 0.11, respectively. Year-class data (Figure 12.4) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 1, which corresponds to the 2021 year-class for Summer Flounder caught in 2022. Summer flounder in the sample in 2022 was dominated by the year classes of 2016, 2017, 2018, and 2019 with 14%, 19%, 19%, and 16%, respectively. The sex ratio of male to female was 1:1.52 for the ocean fish.

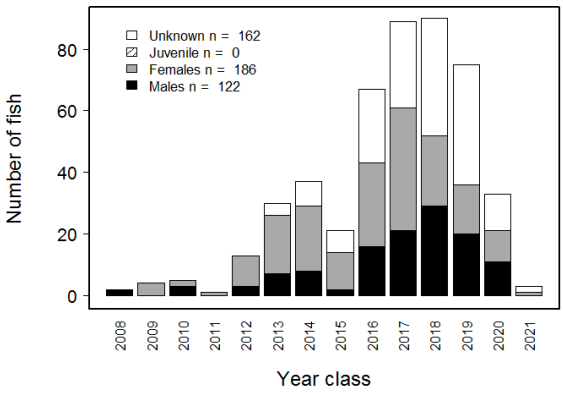


Figure 12.4: Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2022. Distribution is broken down by. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

12.3.3 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 12.7) and ocean fish (Table 12.8) using scale ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

12.3.4 Reading Precision

12.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 425 Tautog collected in 2022. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 80% and a CV of 3.6% (test of symmetry: $\chi^2 = 3.3$, $df = 5$, $P = 0.6487$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 92% and a CV of 1.3% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89% (1 year or less agreement of 100%) and a CV of 1.7% (test of symmetry: $\chi^2 = 23.3$, $df = 10$, $P =$

0.0096) (Figure 12.5).

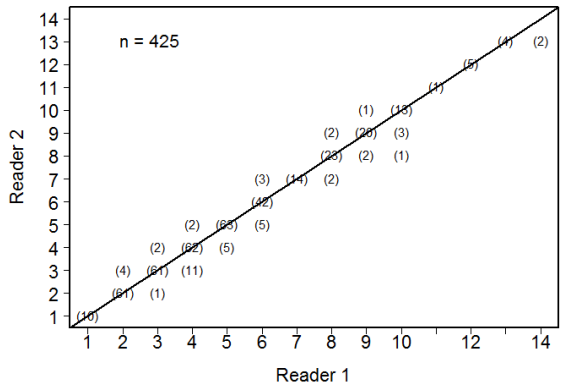


Figure 12.5: Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 94% with ages of fish aged in 2000 with a CV of 1.3% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). Reader 2 had an agreement of 94% with a CV of 1.3% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$).

12.3.4.2 *Scales*

Reader 1 and Reader 2 aged the scales of 839 Summer Flounder collected in 2022. Reader 1 had moderate self-precision and Read 2 had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 58% (1 year or less agreement of 94%) and a CV of 7.4% (test of symmetry: $\chi^2 = 11.2$, $df = 10$, $P = 0.3422$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% (1 year or less agreement of 98%) and a CV of 1.3% (test of symmetry: $\chi^2 = 6$, $df = 5$, $P = 0.3062$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 68% (1 year or less agreement of 93%) and a CV of 7.1% (test of symmetry: $\chi^2 = 68$, $df = 26$, $P < 0.0001$) (Figure 12.6).

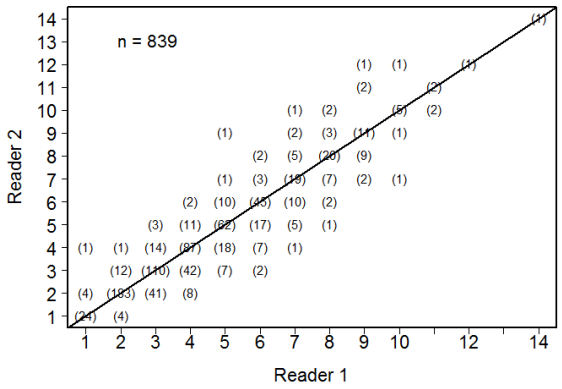


Figure 12.6: Between-reader comparison of scale age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 74% (1 year or less agreement of 100%) with ages of fish aged in 2000 with a CV of 5.5% (test of symmetry: $\chi^2 = 8.2$, $df = 6$, $P = 0.2238$). Reader 2 had an agreement of 88% (1 year or less agreement of 100%) with a CV of 2.5% (test of symmetry: $\chi^2 = 6$, $df = 3$, $P = 0.1116$).

12.3.5 Comparison of Scale and Otolith Ages

We aged 419 pairs of Summer Flounder scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 34$, $df = 33$, $P = 0.4218$) with an average CV of 8.6%. There was an agreement of 58% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 23.9% and 18.6% of the fish, respectively (Figure 12.7). There was also little evidence of bias between otolith and scale ages using an age bias plot (Figure 12.8), with no trend of either over-ageing younger or under-ageing older fish.

12.4 RECOMMENDATIONS

Atlantic States Marine Fisheries Commission held a QAQC ageing workshop in St. Pe-

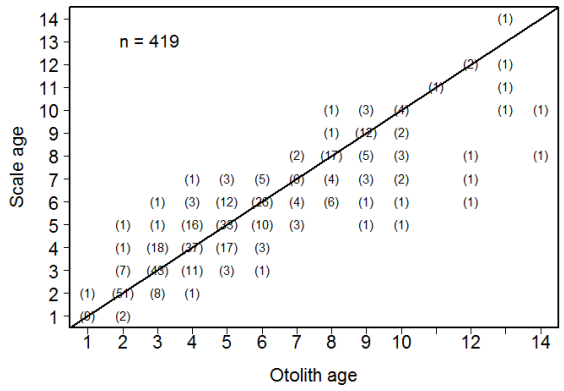


Figure 12.7: Comparison of scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish

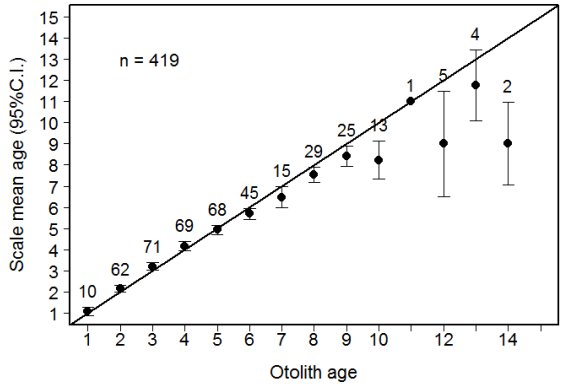


Figure 12.8: Age-bias plot for Summer Flounder scale and otolith age estimates in 2022. The number above the upper CI bar is number of fish.

tersburg, Florida, in March of 2019 (ASMFC 2019). The workshop recommended that summer flounder should be aged using otoliths, not scales, when possible.

Table 12.1: Number of bay Summer Flounder collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
13 - 13.99	5	2	2	3
14 - 14.99	75	95	81	0
15 - 15.99	63	83	66	0
16 - 16.99	47	64	50	0
17 - 17.99	43	66	45	0
18 - 18.99	34	43	43	0
19 - 19.99	27	36	36	0
20 - 20.99	24	31	31	0
21 - 21.99	13	14	14	0
22 - 22.99	5	5	5	0
23 - 23.99	5	0	0	5
24 - 24.99	5	1	1	4
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	1	1	4
Totals	381	441	375	46

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Table 12.2: CV for each age estimated based on ageing the total of 381 bay Summer Flounder in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Summer Flounder collected from 2016 to 2020.

Age	CV	Percent
0	>0.25	0.12
1	0.17	8.38
2	0.06	39.46
3	0.09	25.15
4	0.13	14.43
5	0.18	7.64
6	>0.25	3.33
7	>0.25	1.11
8	>0.25	0.31
9	>0.25	0.06

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Table 12.3: Number of ocean Summer Flounder collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
13 - 13.99	5	1	1	4
14 - 14.99	38	44	43	0
15 - 15.99	57	90	59	0
16 - 16.99	58	87	58	0
17 - 17.99	53	64	64	0
18 - 18.99	41	39	39	2
19 - 19.99	30	27	27	3
20 - 20.99	25	22	22	3
21 - 21.99	22	16	16	6
22 - 22.99	24	26	26	0
23 - 23.99	24	16	16	8
24 - 24.99	21	18	18	3
25 - 25.99	19	18	18	1
26 - 26.99	15	16	16	0
27 - 27.99	14	12	12	2
28 - 28.99	10	15	15	0
29 - 29.99	7	9	9	0
30 - 30.99	5	5	5	0
31 - 31.99	5	4	4	1
32 - 32.99	5	2	2	3
Totals	478	531	470	36

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Table 12.4: CV for each age estimated based on ageing the total of 478 ocean Summer Flounder in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Summer Flounder collected from 2016 to 2020.

Age	CV	Percent
0	>0.25	0.04
1	>0.25	2
2	0.15	8.25
3	0.1	16.28
4	0.09	19.98
5	0.1	17.18
6	0.11	15.05
7	0.14	9.57
8	0.19	5.44
9	0.24	3.06
10	>0.25	1.7
11	>0.25	1.02
12	>0.25	0.34
13	>0.25	0.04
14	>0.25	0.04

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Table 12.5: The number of Summer Flounder assigned to each total length-at-age category for 375 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2022.

	Age								
Interval	1	2	3	4	5	6	7	8	Totals
13 - 13.99	1	0	1	0	0	0	0	0	2
14 - 14.99	16	53	11	1	0	0	0	0	81
15 - 15.99	1	56	9	0	0	0	0	0	66
16 - 16.99	0	31	13	5	1	0	0	0	50
17 - 17.99	3	23	13	5	0	1	0	0	45
18 - 18.99	0	7	15	15	5	1	0	0	43
19 - 19.99	0	12	11	11	2	0	0	0	36
20 - 20.99	0	9	7	8	3	4	0	0	31
21 - 21.99	0	1	8	3	2	0	0	0	14
22 - 22.99	0	1	1	1	1	0	1	0	5
24 - 24.99	0	0	0	1	0	0	0	0	1
30 - 30.99	0	0	0	0	0	0	0	1	1
Totals	21	193	89	50	14	6	1	1	375

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Table 12.6: The number of Summer Flounder assigned to each total length-at-age category for 470 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2022.

	Age														
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Totals
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
14 - 14.99	1	7	20	6	8	1	0	0	0	0	0	0	0	0	43
15 - 15.99	0	13	16	17	8	4	0	1	0	0	0	0	0	0	59
16 - 16.99	2	5	11	15	11	8	1	2	2	0	0	1	0	0	58
17 - 17.99	0	3	15	20	6	10	3	2	2	2	0	1	0	0	64
18 - 18.99	0	4	8	11	11	0	0	3	1	0	0	0	0	1	39
19 - 19.99	0	0	3	8	9	4	0	1	0	1	0	1	0	0	27
20 - 20.99	0	0	0	5	10	5	0	1	1	0	0	0	0	0	22
21 - 21.99	0	0	0	1	5	6	2	1	1	0	0	0	0	0	16
22 - 22.99	0	0	1	6	9	7	2	0	0	0	0	0	0	1	26
23 - 23.99	0	0	0	1	2	11	1	1	0	0	0	0	0	0	16
24 - 24.99	0	0	1	0	4	7	3	2	1	0	0	0	0	0	18
25 - 25.99	0	0	0	0	2	3	3	5	4	1	0	0	0	0	18
26 - 26.99	0	0	0	0	3	1	3	5	3	1	0	0	0	0	16
27 - 27.99	0	0	0	0	1	0	2	4	3	1	0	1	0	0	12
28 - 28.99	0	0	0	0	0	0	0	4	6	3	1	0	1	0	15
29 - 29.99	0	0	0	0	0	0	1	2	3	2	0	0	1	0	9
30 - 30.99	0	0	0	0	0	0	0	2	0	1	0	1	1	0	5
31 - 31.99	0	0	0	0	0	0	0	1	3	0	0	0	0	0	4
32 - 32.99	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2
Totals	3	33	75	90	89	67	21	37	30	13	1	5	4	2	470

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Table 12.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Chesapeake Bay, Virginia during 2022.

	Age							
Interval	1	2	3	4	5	6	7	8
13 - 13.99	0.5	0	0.5	0	0	0	0	0
14 - 14.99	0.2	0.65	0.14	0.01	0	0	0	0
15 - 15.99	0.02	0.85	0.14	0	0	0	0	0
16 - 16.99	0	0.62	0.26	0.1	0.02	0	0	0
17 - 17.99	0.07	0.51	0.29	0.11	0	0.02	0	0
18 - 18.99	0	0.16	0.35	0.35	0.12	0.02	0	0
19 - 19.99	0	0.33	0.31	0.31	0.06	0	0	0
20 - 20.99	0	0.29	0.23	0.26	0.1	0.13	0	0
21 - 21.99	0	0.07	0.57	0.21	0.14	0	0	0
22 - 22.99	0	0.2	0.2	0.2	0.2	0	0.2	0
24 - 24.99	0	0	0	1	0	0	0	0
30 - 30.99	0	0	0	0	0	0	0	1

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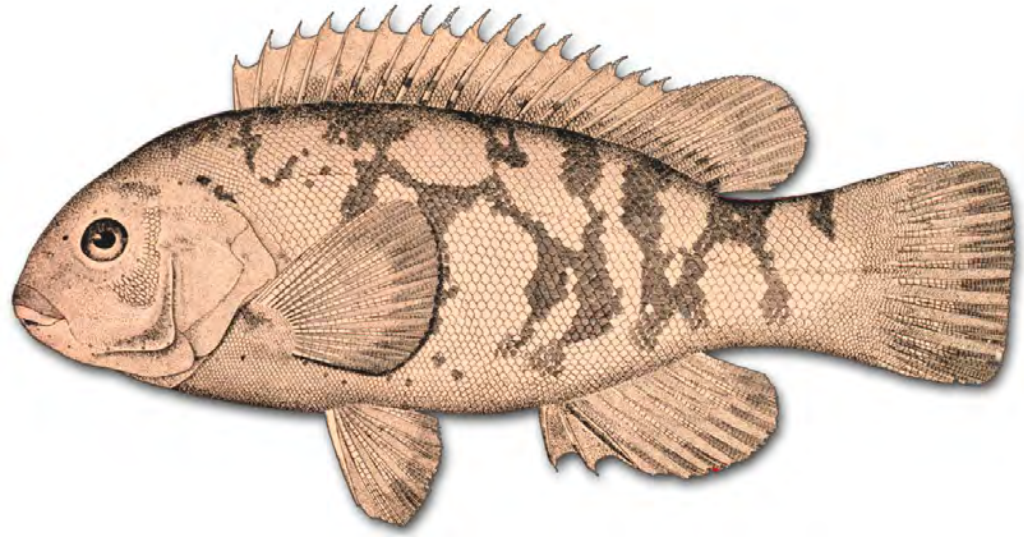
Table 12.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Virginia waters of the Atlantic Ocean during 2022.

	Age													
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0.02	0.16	0.47	0.14	0.19	0.02	0	0	0	0	0	0	0	0
15 - 15.99	0	0.22	0.27	0.29	0.14	0.07	0	0.02	0	0	0	0	0	0
16 - 16.99	0.03	0.09	0.19	0.26	0.19	0.14	0.02	0.03	0.03	0	0	0.02	0	0
17 - 17.99	0	0.05	0.23	0.31	0.09	0.16	0.05	0.03	0.03	0.03	0	0.02	0	0
18 - 18.99	0	0.1	0.21	0.28	0.28	0	0	0.08	0.03	0	0	0	0	0.03
19 - 19.99	0	0	0.11	0.3	0.33	0.15	0	0.04	0	0.04	0	0.04	0	0
20 - 20.99	0	0	0	0.23	0.45	0.23	0	0.05	0.05	0	0	0	0	0
21 - 21.99	0	0	0	0.06	0.31	0.38	0.12	0.06	0.06	0	0	0	0	0
22 - 22.99	0	0	0.04	0.23	0.35	0.27	0.08	0	0	0	0	0	0	0.04
23 - 23.99	0	0	0	0.06	0.12	0.69	0.06	0.06	0	0	0	0	0	0
24 - 24.99	0	0	0.06	0	0.22	0.39	0.17	0.11	0.06	0	0	0	0	0
25 - 25.99	0	0	0	0	0.11	0.17	0.17	0.28	0.22	0.06	0	0	0	0
26 - 26.99	0	0	0	0	0.19	0.06	0.19	0.31	0.19	0.06	0	0	0	0
27 - 27.99	0	0	0	0	0.08	0	0.17	0.33	0.25	0.08	0	0.08	0	0
28 - 28.99	0	0	0	0	0	0	0	0.27	0.4	0.2	0.07	0	0.07	0
29 - 29.99	0	0	0	0	0	0	0.11	0.22	0.33	0.22	0	0	0.11	0
30 - 30.99	0	0	0	0	0	0	0	0.4	0	0.2	0	0.2	0.2	0
31 - 31.99	0	0	0	0	0	0	0	0.25	0.75	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0

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Chapter 13

TAUTOG *Tautoga onitis*



13.1 INTRODUCTION

We aged a total of 181 Tautog *Tautoga onitis*, collected by the VMRC's Biological Sampling Program in 2022. Of 181 aged fish, 174 and 7 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average age for the bay fish was 5.3 years with a standard deviation of 2.5 and a standard error of 0.19. Eleven age classes (2 to 9, 11, 14, and 16) were represented in the bay fish, comprising fish from the 2006, 2008, 2011, and 2013 to 2020 year classes. The bay fish sample in 2022 was dominated by the year classes of 2015 and 2019 with 20% and 25%, respectively. Only 7 ocean fish were collected, 4, 7 to 8, 14, 21 to 22, and 24 years old, and in the year class of 1998, 2000 to 2001, 2008, 2014 to 2015, and 2018.

Of the 181 samples aged, 178 fish were aged with all three structures, otoliths, opercula, and spines. In addition, 2 fish were aged with otoliths and opercula. As a result, we were able to examine the precisions between otolith- and operculum-ages (180 pairs) and between otolith- and spine-ages (178 pairs), respectively.

13.2 METHODS

13.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Tautog collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Tautog in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Tautog used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Tautog collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

13.2.2 Handling of Collection

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

13.2.3 Hardpart Preparation

13.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Tautog otoliths for age determination. Otolith preparation began by placing both whole otoliths in a ceramic "Coors" spot

plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved, the baked otoliths were embedded in epoxy resin seperatly with the distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

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

13.2.3.2 *Opercula*

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

Click here to obtain the protocol at the VMRC website on how to prepare opercula for ageing Tautog.

13.2.3.3 *Spines*

Following the instructions in the ASMFC Ageing Workshop and the methods in Elzey and Trull (2016), we started to age Tautog collected in 2022 uisng their pelvic fin spines (hereafter, referred to at spines). The spines were boiled for several minutes to remove any skin and connective tissue. After boiling, spines were stored in labeled coin envelopes for at least 24 hours to ensure the spines were fully dry. Once dry, the spines were embedded in epoxy resin and allowed to cure overnight. At least three thin-sections were removed from the resin block using a Buhler Isomet low-speed saw equipped with four, 4 inch diameter diamond wafering blades each separated by a 0.75 mm stainless steel spacer. The sections were then mounted to labeled glass slides in order with the first section, closest to the body of the fish, on the right and affixed with Flo-texx mounting medium.

13.2.4 Readings

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

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

added to the notation.

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

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

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

13.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 13.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

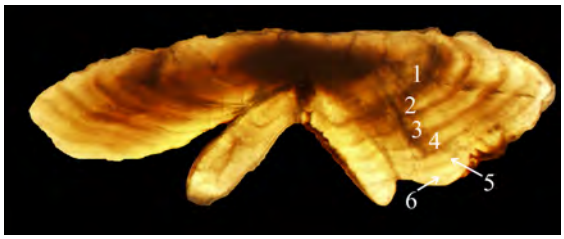


Figure 13.1: Otolith thin-section of 6 year-old Tautog

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

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

13.2.4.2 *Opercula*

Opercula were aged on a light table with no magnification (Figure 13.2). Tautog opercula are also considered to have a deposition period of May through July (Hostetter and Munroe 1993), and age class assignment using these hard-parts is conducted in the same way as otoliths.

13.2.4.3 *Spines*

All spine thin-sections were aged using an Olympus BX41 compound microscope (Figure 13.3). Since there were at least three sections per slide, Reader 1 and 2 will mark their sections with a red and black dot, respectively, when they chose different sections for their age

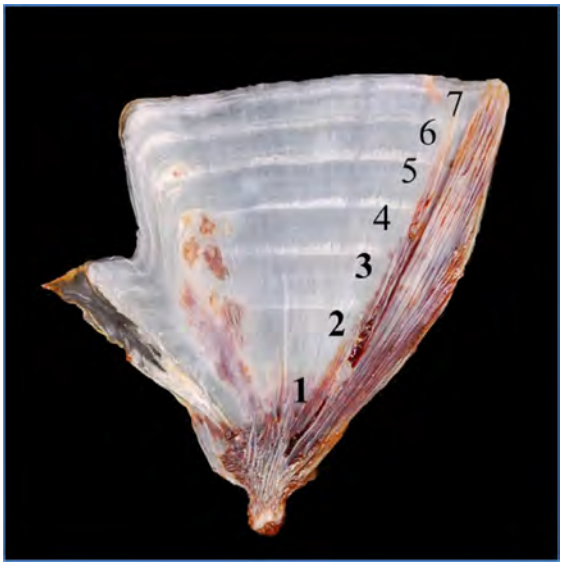


Figure 13.2: Operculum of a 7 year-old Tautog

estimates. If there was a disagreement, both marked sections were reviewed to determine a final age.

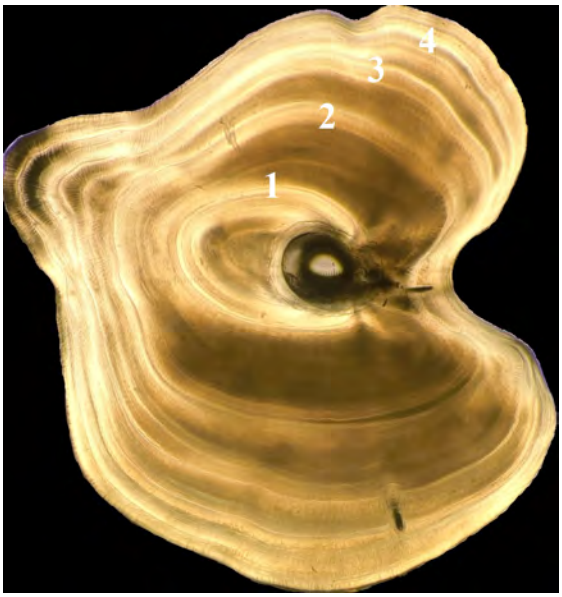


Figure 13.3: Spine of a 4 year-old Tautog

13.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; 4) between operculum and otoliths ages; and 5) between spine and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and opercula randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

13.3 RESULTS

13.3.1 Sample Size

The sample sizes are estimated for Tautog in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lenghts and any combinations of three hardparts (Otoliths, opercula, and spines).

13.3.1.1 *Chesapeake Bay*

We estimated a sample size of 452 bay Tautog in 2022, ranging in length interval from 8 to 26 inches (Table 13.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 4 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 13.2). We aged all the fish with otoliths, opercula, and spines (171 fish). We aged 2 fish with both otoliths and opercula. We aged 1 fish with opercula and spines. As a result, we aged all of 174 fishcollected by VMRC in Chesapeake Bay in 2022. We fell short in our over-all collections for this optimal length-class sampling estimate by 293 fish. We were

short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

13.3.1.2 *Atlantic Ocean*

We estimated a sample size of 454 ocean Tautog in 2022, ranging in length interval from 8 to 30 inches (Table 13.3). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 9% for the major age of Age 5 to the *CV* of larger than 25% for the multiple minor ages of the ocean fish (Table 13.4). We aged all the fish with otoliths, opercula, and spines (7 fish). As a result, we aged all of 7 fish collected by VMRC in Virginia waters of Atlantic Ocean in 2022. We fell short in our over-all collections for this optimal length-class sampling estimate by 447 fish. We were short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

13.3.2 Year Class

Year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The otolith ages are always the primary ages. When the otolith ages were not available, the operculum ages were used, followed by the spine ages.

13.3.2.1 *Chesapeake Bay*

Of the 174 bay Tautog aged, 11 age classes (2 to 9, 11, 14, and 16) were represented (Table 13.5). The average age for the sample was 5.3 years. The standard deviation and standard error were 2.5 and 0.19, respectively. Year-class data (Figure 13.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2020 year-class for Tautog caught in 2022. Tautog in the sample in 2022 was dominated by the year classes of 2015 and 2019 with 20% and 25%, respectively. The sex ratio of male to female was 1:1.36 for the bay fish.

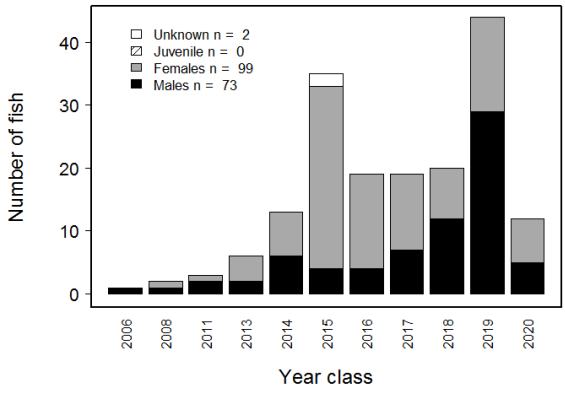


Figure 13.4: Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

13.3.2.2 *Atlantic Ocean*

Only 7 ocean fish was collected, 4, 7 to 8, 14, 21 to 22, and 24 years old, and in the year class of 1998, 2000 to 2001, 2008, 2014 to 2015, and 2018 (Table 13.6).

13.3.3 Age-Length-Key (ALK)

We developed an age-length-key for bay fish (Table 13.7) using all the aged fish described in Section Sample Size. No ALK was developed for the ocean tautog because there was only 7 ocean fish collected and aged in 2022. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

13.3.4 Reading Precision

13.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 180 Tautog collected in 2022. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a CV of 0.1% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 96% and a CV of 0.4% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 93% (1 year or less agreement of 99%) and a CV of 0.7% (test of symmetry: $\chi^2 = 9.3$, $df = 10$, $P = 0.5008$) (Figure 13.5).

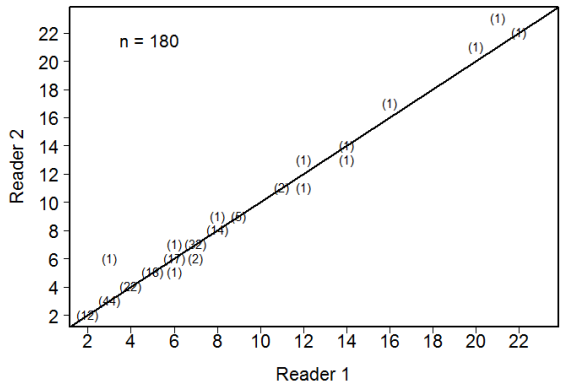


Figure 13.5: Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlatic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 92% with ages of fish aged in 2000 with a CV of 1% (test of symmetry: $\chi^2 = 4$, $df = 2$, $P = 0.1353$). Reader 2 had an agreement of 94% with a CV of 0.7% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$).

13.3.4.2 *Opercula*

Reader 1 and Reader 2 aged the opercula of 181 Tautog collected in 2022. Reader 1 had moderate self-precision and Read 2 had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 70% (1 year or less agreement of 92%) and a CV of 5.7% (test of symmetry: $\chi^2 = 12.3$, $df = 11$, $P = 0.3391$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 82% (1 year or less agreement of 98%) and a CV of 2.1% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 70% (1 year or less agreement of 94%) and a CV of 4.8% (test of symmetry: $\chi^2 = 19.8$, $df = 20$, $P = 0.4723$) (Figure 13.6).

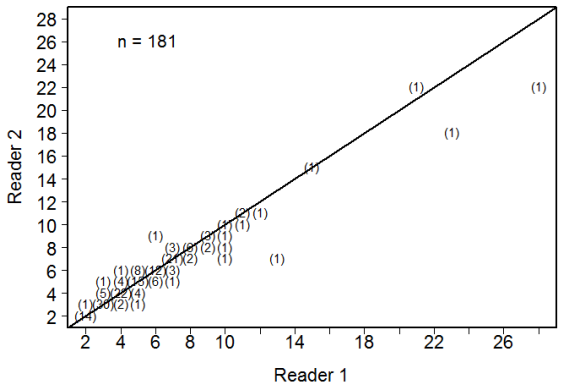


Figure 13.6: Between-reader comparison of operculum age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 56% (1 year or less agreement of 98%) with ages of fish aged in 2000 with a CV of 6.3% (test of symmetry: $\chi^2 = 4.5$, $df = 9$, $P = 0.8729$). Reader 2 had an agreement of 76% (1 year or less agreement of 98%) with a CV of 3.7% (test of symmetry: $\chi^2 = 9.3$, $df = 6$, $P = 0.1557$).

13.3.4.3 *Spines*

Reader 1 and Reader 2 aged the spines of 179 Tautog collected in 2022. Reader 1 had low

self-precision and Read 2 had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 48% (1 year or less agreement of 72%) and a CV of 13.2% (test of symmetry: $\chi^2 = 11.9$, $df = 15$, $P = 0.6891$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 54% (1 year or less agreement of 84%) and a CV of 8.3% (test of symmetry: $\chi^2 = 14.7$, $df = 13$, $P = 0.3286$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 51% (1 year or less agreement of 79%) and a CV of 9.5% (test of symmetry: $\chi^2 = 36.3$, $df = 27$, $P = 0.1094$) (Figure 13.7).

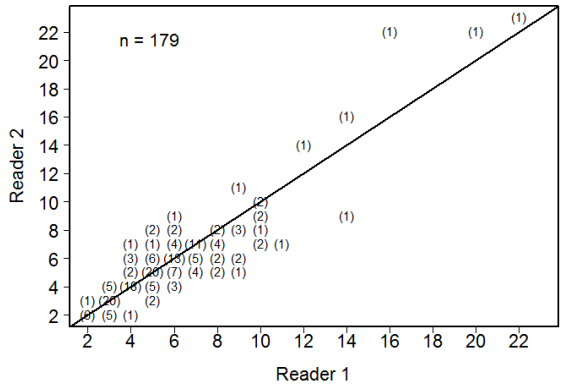


Figure 13.7: Between-reader comparison of spine age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

We didn't conduct time-series comparison in spine ageing because it was our first year to age Tautog spines and we didn't have any time-series set of spine ages to compare. We will start to do the time-series comparison in spine ageing using the spine ages collected in 2022 as the reference.

13.3.5 Comparisons

13.3.5.1 *Operculum vs otolith ages*

We aged 180 pairs of Tautog opercula and otoliths. There was an evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 38.3$, $df = 22$, $P = 0.0169$) with an average CV of 5.9%. There was an agreement of 64% between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 25.6% and 10.6% of the fish, respectively (Figure 13.8). There was also an evidence of bias between otolith and operculum ages using an age bias plot (Figure 13.9), with operculum generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

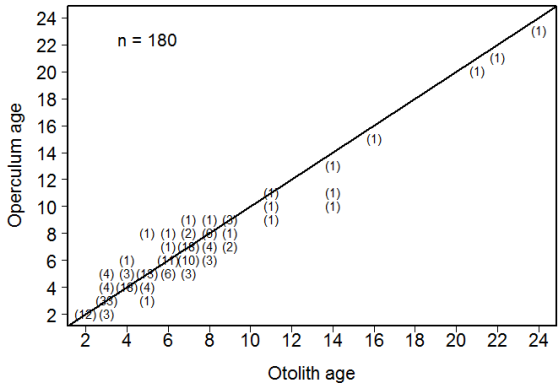


Figure 13.8: Comparison of operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

13.3.5.2 *Spine vs otolith ages*

We aged 178 pairs of Tautog spines and otoliths. There was an evidence of systematic disagreement between otolith and spine ages (test of symmetry: $\chi^2 = 36.2$, $df = 23$, $P = 0.0396$) with an average CV of 11.1%. There was an agreement of 44% between spine and otoliths ages whereas spines were assigned a lower and higher age than otoliths for 27.5% and 28.7% of the fish, respectively (Figure

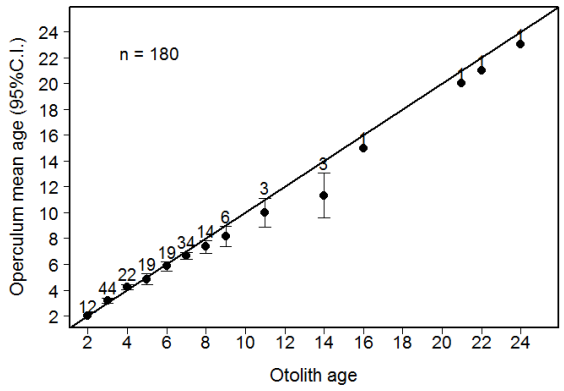


Figure 13.9: Age-bias plot for Tautog operculum and otolith age estimates in 2022. The number above the upper CI bar is number of fish.

13.10). There was also an evidence of bias between otolith and spine ages using an age bias plot (Figure 13.11), with spine generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

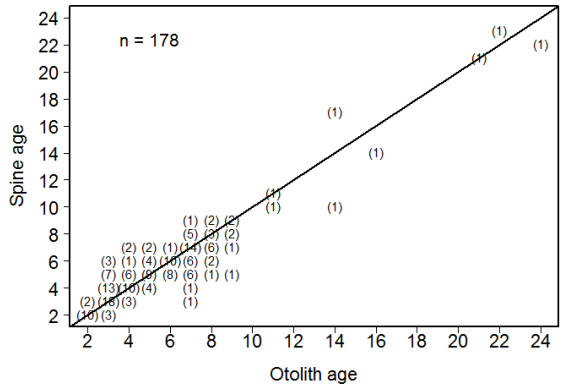


Figure 13.10: Comparison of spine and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

13.4 RECOMMENDATIONS

Atlantic States Marine Fisheries Commission held a QAQC ageing workshop in St. Petersburg, Florida, in March of 2023. The workshop recommended that otolith ages should be used as the primary age for Tautog when possible. Our results also indicates that the precision on otolith ageing is the highest among the three hardparts we aged, supporting the ASMFC recommendation.

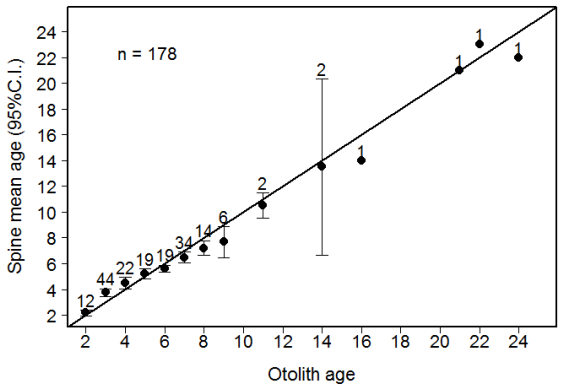


Figure 13.11: Age-bias plot for Tautog spine and otolith age estimates in 2022. The number above the upper CI bar is number of fish.

Table 13.1: Number of bay Tautog collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	5	5	5	0
11 - 11.99	5	11	11	0
12 - 12.99	6	15	15	0
13 - 13.99	37	14	14	23
14 - 14.99	75	22	22	53
15 - 15.99	88	33	33	55
16 - 16.99	75	32	32	43
17 - 17.99	57	23	23	34
18 - 18.99	34	7	7	27
19 - 19.99	21	5	5	16
20 - 20.99	9	3	3	6
21 - 21.99	5	3	3	2
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	1	1	4
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
Totals	452	174	174	293

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Table 13.2: CV for each age estimated based on ageing the total of 452 bay Tautog in 2022. 'Percent' is the percentage of an age in the pooled age-length data of bay Tautog collected from 2016 to 2020.

Age	CV	Percent
1	>0.25	1.16
2	0.17	6.37
3	0.1	18.51
4	0.09	21.88
5	0.1	20.46
6	0.12	12.76
7	0.18	6.67
8	0.21	4.86
9	>0.25	2.87
10	>0.25	1.95
11	>0.25	0.7
12	>0.25	0.66
13	>0.25	0.59
14	>0.25	0.35
15	>0.25	0.11
16	>0.25	0.07
17	>0.25	0.02
18	>0.25	0.02

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Table 13.3: Number of ocean Tautog collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	5	0	0	5
11 - 11.99	8	0	0	8
12 - 12.99	5	0	0	5
13 - 13.99	32	0	0	32
14 - 14.99	46	0	0	46
15 - 15.99	57	1	1	56
16 - 16.99	62	0	0	62
17 - 17.99	43	0	0	43
18 - 18.99	36	0	0	36
19 - 19.99	28	1	1	27
20 - 20.99	25	0	0	25
21 - 21.99	18	0	0	18
22 - 22.99	17	1	1	16
23 - 23.99	14	0	0	14
24 - 24.99	9	1	1	8
25 - 25.99	8	0	0	8
26 - 26.99	9	2	2	7
27 - 27.99	7	0	0	7
28 - 28.99	5	1	1	4
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	454	7	7	447

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Table 13.4: CV for each age estimated based on ageing the total of 454 ocean Tautog in 2022. 'Percent' is the percentage of an age in the pooled age-length data of ocean Tautog collected from 2016 to 2020.

Age	CV	Percent
2	>0.25	1.99
3	0.15	8.54
4	0.11	13.92
5	0.09	20.47
6	0.1	17.54
7	0.15	9.47
8	0.16	8.19
9	0.23	3.86
10	>0.25	2.69
11	>0.25	2.34
12	>0.25	1.4
13	>0.25	2.34
14	>0.25	1.29
15	>0.25	1.52
16	>0.25	1.29
17	>0.25	0.47
18	>0.25	0.7
20	>0.25	0.47
21	>0.25	0.23
22	>0.25	0.23
23	>0.25	0.47
24	>0.25	0.12
27	>0.25	0.23
30	>0.25	0.12
31	>0.25	0.12

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Table 13.5: The number of Tautog assigned to each total length-at-age category for 174 fish sampled for both otolith and operculum age determination in Chesapeake Bay, Virginia during 2022.

	Age											
Interval	2	3	4	5	6	7	8	9	11	14	16	Totals
10 - 10.99	4	1	0	0	0	0	0	0	0	0	0	5
11 - 11.99	5	5	0	0	0	1	0	0	0	0	0	11
12 - 12.99	2	12	1	0	0	0	0	0	0	0	0	15
13 - 13.99	1	11	0	2	0	0	0	0	0	0	0	14
14 - 14.99	0	9	5	1	4	3	0	0	0	0	0	22
15 - 15.99	0	3	8	7	5	8	1	1	0	0	0	33
16 - 16.99	0	3	3	6	6	11	2	1	0	0	0	32
17 - 17.99	0	0	2	2	2	9	5	2	1	0	0	23
18 - 18.99	0	0	1	0	0	2	1	1	1	1	0	7
19 - 19.99	0	0	0	1	1	1	2	0	0	0	0	5
20 - 20.99	0	0	0	0	1	0	0	1	1	0	0	3
21 - 21.99	0	0	0	0	0	0	2	0	0	1	0	3
24 - 24.99	0	0	0	0	0	0	0	0	0	0	1	1
Totals	12	44	20	19	19	35	13	6	3	2	1	174

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Table 13.6: The number of Tautog assigned to each total length-at-age category for 7 fish sampled for both otolith and operculum age determination in Virginia waters of Atlantic ocean during 2022.

	Age							
Interval	4	7	8	14	21	22	24	Totals
15 - 15.99	1	0	0	0	0	0	0	1
19 - 19.99	0	0	1	0	0	0	0	1
22 - 22.99	0	1	0	0	0	0	0	1
24 - 24.99	0	0	0	1	0	0	0	1
26 - 26.99	0	0	0	0	1	1	0	2
28 - 28.99	0	0	0	0	0	0	1	1
Totals	1	1	1	1	1	1	1	7

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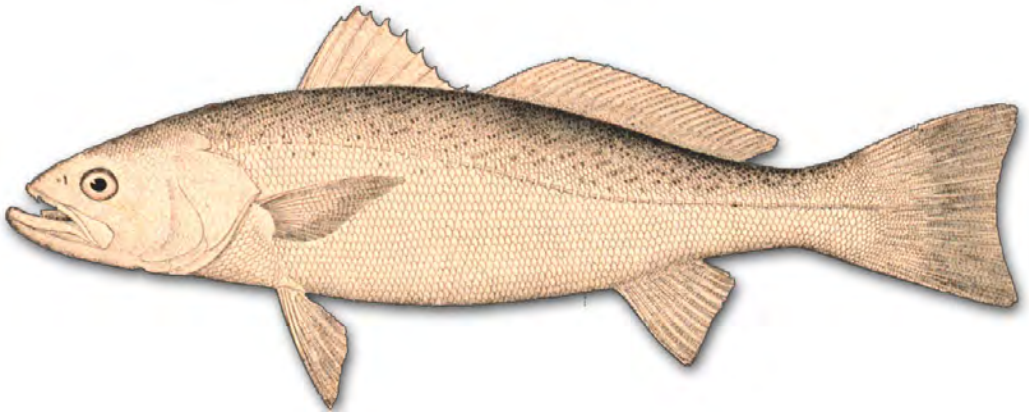
Table 13.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and operculum ages for Tautog sampled in Chesapeake Bay, Virginia during 2022.

	Age										
Interval	2	3	4	5	6	7	8	9	11	14	16
10 - 10.99	0.8	0.2	0	0	0	0	0	0	0	0	0
11 - 11.99	0.45	0.45	0	0	0	0.09	0	0	0	0	0
12 - 12.99	0.13	0.8	0.07	0	0	0	0	0	0	0	0
13 - 13.99	0.07	0.79	0	0.14	0	0	0	0	0	0	0
14 - 14.99	0	0.41	0.23	0.05	0.18	0.14	0	0	0	0	0
15 - 15.99	0	0.09	0.24	0.21	0.15	0.24	0.03	0.03	0	0	0
16 - 16.99	0	0.09	0.09	0.19	0.19	0.34	0.06	0.03	0	0	0
17 - 17.99	0	0	0.09	0.09	0.09	0.39	0.22	0.09	0.04	0	0
18 - 18.99	0	0	0.14	0	0	0.29	0.14	0.14	0.14	0.14	0
19 - 19.99	0	0	0	0.2	0.2	0.2	0.4	0	0	0	0
20 - 20.99	0	0	0	0	0.33	0	0	0.33	0.33	0	0
21 - 21.99	0	0	0	0	0	0	0.67	0	0	0.33	0
24 - 24.99	0	0	0	0	0	0	0	0	0	0	1

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Chapter 14

WEAKFISH *Cynoscion regalis*



14.1 INTRODUCTION

We aged a total of 235 Weakfish *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2022. The Weakfish ages ranged from 1 to 5 years old with an average age of 2.4, a standard deviation of 0.8, and a standard error of 0.05. Five age classes (1 to 5) were represented, comprising fish of the 2017 to 2021 year-classes. The sample was dominated by fish from the year-class of 2020 with 57.5%.

14.2 METHODS

14.2.1 Sample Size for Ageing

We estimated sample size for ageing Weakfish in 2022 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Weakfish in 2022; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Weakfish used by VMRC to estimate length distribution of the catches from 2016 to 2020. θ_a, V_a, and B_a were calculated using pooled age-length data of Weakfish collected from 2016 to 2020 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2016 to 2020 catch. A_l is number of fish to be aged for length interval l in 2022.

14.2.2 Handling of Collections

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

14.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Lowerre-Barbieri et al. (1994) with a few modifications. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

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

14.2.4 Readings

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

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

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

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

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 14.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

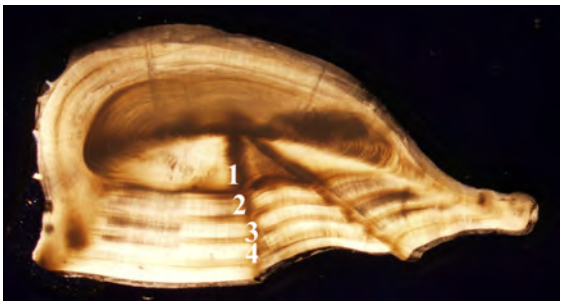


Figure 14.1: Otolith thin-section of 4 year-old Weakfish

All samples were aged by two readers in chronological order, based on collection date,

without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, 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. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

14.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

14.3 RESULTS

14.3.1 Sample Size

We estimated a sample size of 326 for ageing Weakfish in 2022, ranging in length interval from 4 to 34 inches (Table 14.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for Age 2 to the CV of larger than 25% for the multiple minor ages (Table 14.2). In 2022, we aged 235 of 245 Weakfish (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 107 fish. We were short of some fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would possibly be influenced significantly.

14.3.2 Year Class

Of the 235 fish aged with otoliths, 5 age classes (1 to 5) were represented (Table 14.3). The average age was 2.4 years, and the standard deviation and standard error were 0.8 and 0.05, respectively. Year-class data show that the fishery was comprised of 5 year-classes: fish from the 2017 to 2021 year-classes, with fish primarily from the year-class of 2020 with 57.5%. The ratio of males to females was 1:4.44 in the sample collected (Figure 14.2).

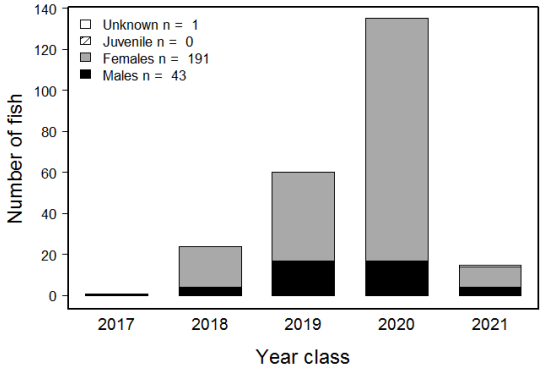


Figure 14.2: Year-class frequency distribution for Weakfish collected for ageing in 2022. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

14.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 14.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-

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

14.3.4 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 100%, and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.15% and a CV of 0.29% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$) (Figure 14.3).

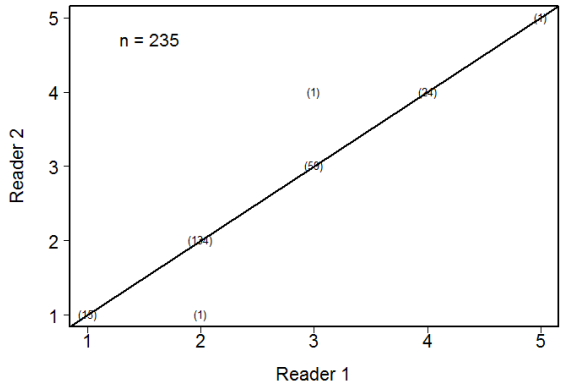


Figure 14.3: Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2022. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with ages of fish aged in 2000 with a CV of 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 98% with a CV of 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

Table 14.1: Number of Weakfish collected and aged in each 1-inch length interval in 2022. 'Target' represents the sample size for ageing estimated for 2022, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
6 - 6.99	5	0	0	5
7 - 7.99	5	1	1	4
8 - 8.99	9	15	15	0
9 - 9.99	29	30	30	0
10 - 10.99	48	40	40	8
11 - 11.99	38	34	34	4
12 - 12.99	30	33	33	0
13 - 13.99	20	30	20	0
14 - 14.99	15	19	19	0
15 - 15.99	18	15	15	3
16 - 16.99	14	7	7	7
17 - 17.99	9	11	11	0
18 - 18.99	6	5	5	1
19 - 19.99	5	1	1	4
20 - 20.99	5	0	0	5
21 - 21.99	5	1	1	4
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	1	1	4
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	2	2	3
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	326	245	235	107

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Table 14.2: CV for each age estimated based on ageing the total of 326 Weakfish in 2022. 'Percent' is the percentage of an age in the pooled age-length data of Weakfish collected from 2016 to 2020.

Age	CV	Percent
0	>0.25	0.37
1	0.11	16.04
2	0.06	47.76
3	0.07	30.82
4	0.22	4.63
5	>0.25	0.22
6	>0.25	0.15

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Table 14.3: The number of Weakfish assigned to each total length-at-age category for 235 fish sampled for otolith age determination in Virginia during 2022.

	Age					
Interval	1	2	3	4	5	Totals
7 - 7.99	1	0	0	0	0	1
8 - 8.99	2	13	0	0	0	15
9 - 9.99	2	28	0	0	0	30
10 - 10.99	7	33	0	0	0	40
11 - 11.99	1	23	10	0	0	34
12 - 12.99	2	15	14	2	0	33
13 - 13.99	0	2	13	5	0	20
14 - 14.99	0	10	4	5	0	19
15 - 15.99	0	5	7	3	0	15
16 - 16.99	0	2	3	2	0	7
17 - 17.99	0	1	6	4	0	11
18 - 18.99	0	2	3	0	0	5
19 - 19.99	0	1	0	0	0	1
21 - 21.99	0	0	0	1	0	1
24 - 24.99	0	0	0	1	0	1
27 - 27.99	0	0	0	1	1	2
Totals	15	135	60	24	1	235

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Table 14.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Weakfish sampled for age determination in Virginia during 2022.

	Age				
Interval	1	2	3	4	5
7 - 7.99	1	0	0	0	0
8 - 8.99	0.13	0.87	0	0	0
9 - 9.99	0.07	0.93	0	0	0
10 - 10.99	0.17	0.82	0	0	0
11 - 11.99	0.03	0.68	0.29	0	0
12 - 12.99	0.06	0.45	0.42	0.06	0
13 - 13.99	0	0.1	0.65	0.25	0
14 - 14.99	0	0.53	0.21	0.26	0
15 - 15.99	0	0.33	0.47	0.2	0
16 - 16.99	0	0.29	0.43	0.29	0
17 - 17.99	0	0.09	0.55	0.36	0
18 - 18.99	0	0.4	0.6	0	0
19 - 19.99	0	1	0	0	0
21 - 21.99	0	0	0	1	0
24 - 24.99	0	0	0	1	0
27 - 27.99	0	0	0	0.5	0.5

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APPENDIX

Analyses and Applications of Black Drum Age and Length Data Collected by Atlantic States between 2008 and 2019

Analyses and Applications of Black Drum Age and Length Data Collected by Atlantic States between 2008 and 2019

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(Working paper)

August 3, 2022

Age Data Working Group
ASMFC Black Drum TC and SASC

Introduction

Black Drum (*Pogonias cromis*) 2015 benchmark stock assessment used three catch-based methods to evaluate Black Drum stock status and estimate biological reference points. One of the reasons for using the data-poor methods is the lack of age-length data and length distribution data, the former represents the relationship between age and length, and the latter represents length distribution of a catch. After the last stock assessment, the state agencies along the east coast have continued to collect age-length and length data from both commercial and recreational fisheries, fishery-independent surveys for multiple years. The primary goal of this study is to find out if the age-length and length data are sufficient enough to provide information for tracking cohort progressions through years, and to update von Bertalanffy growth parameters for age-specific natural mortality estimate. The specific objectives are: 1) evaluate the length data collected by Atlantic states to see if the data from different units (gear, state, region) can be collapsed to increase sample sizes; 2) evaluate the age-length data collected by Atlantic states to see if the data can be used to convert length distribution to age distribution; 3) examine if converted age distributions can track cohort progressions through years; 4) explore the implication of such information in Black Drum stock assessment; 5) fit von Bertalanffy growth model to the age-length data to estimate the growth parameters; 6) discuss the implication of these parameters in estimation of age-specific natural mortality.

Methods

Data collection

Atlantic state agencies collected all the data used in this study. There are three sets of data as follows:

1. Length data: total length and fork length in mm, cm, or inch;
2. Age-length data: otolith age, total and fork length in mm, cm, or inch;
3. Abundance index: Age-0 and Age-1.

Length data

DE, MD, VA, NC, SC, and FL collected either total, folk length or both. Some states collected the data as early as 1980, all the states collected the data to 2020. The data were collected mainly from commercial fisheries and some from recreational fisheries using a variety of gears.

Age-length data

DE, VA, NC, SC, and FL collected the age-length data. FL collected the data as early as 1983 whereas most of states collected the data to present. The fish and carcasses were collected from recreational and commercial fisheries, and fishery-independent surveys, however, mainly from the recreational fisheries. The otoliths were used to estimate ages.

Abundance index

NC provided an abundance index showing year-class strength ranging from 2001 to 2019. SC provided trammel net CPUE for Age-1 ranging from 1991 to 2021.

Data analysis

Length data

All the lengths in cm or inch were converted to mm. We used the fish with both total and folk length to develop a linear model and then used the model to convert folk length to total length for the fish who have only folk length as follows:

1. Assuming that the difference between total and folk length is normally distributed, we used boxplot function boxplot() in R (R Core Team 2021) to identify outliers of the differences, and removed any fish with those outliers;

2. We used the rest fish to develop a linear model, TOTAL = a + b x FOLK, where, FORK, TOTAL, a, and b stand for fork length, total length, intercept, and slope, respectively.

We used boxplot and Tukey test (TukeyHSD() in Package "stats" in R) to examine the differences in mean total length between gears with each state and between states within each gear to explore if we could collapse those units to increase sample sizes of length due to small sample size within some units. Based on the test results we collapse two or more gears and/or states to a fleet. We used the selected length data to make annual 1-inch length interval distributions for further age conversions.

Age-length data

We also standardized the length in the age-length data to total length in mm as described above. We used Kimura likelihood ratio test (Kimura 1980), growthlrt() function in Package "fishmethods" in R) to test differences in von Bertalanffy growth rate between sexes, states, and regions, to explore if we were able to collapse those units to increase the sample sizes of age-length data because it is difficult to collect Black Drum age-length data in general. Because there is no sex information in the age-length data collected from the fishery-independent surveys, we excluded all the fishery-independent surveys from Kimura test. More specific:

1. Assuming no significant difference in Black Drum growth rate between years, or at least no increasing or decreasing trend in their growth through years, we collapse all year data to test;

2. We used boxplot function to remove outliears by sex, state, and region, respectively, before testing the growth rates;

3. We used Kimura likelihood ratio test (fishmethods package in R) to test between sexes, any two states, and two regions (Mid-Atlantic region (DE, MD, and VA) versus South Atlantic region (NC, SC, GA, and FL)).

ALK and Conversion of length to age

Based on the Kimura test results we collapsed certain units to make annual ALKs. Here we included the age-length data from the fishery-independent surveys in the ALKs unlike in the Kimura tests described previously. This is because the fishery-independent data mainly consists of younger fish whereas the fishery-dependent data lacks of younger fish, and the combination of both will make the ALKs more representative of the relationship between age and length in the Black Drum population. Because there were few samples of age-length data before 2008, we removed any years before 2008 for further analysis. As a result, we converted the length distributions to age distributions from 2008 to present. In addition, for demonstration purpose of cohort progressions, we presented the

conversions only from 2008 to 2019, making a 12-panel page (or 12 years in one page). We did the conversions as follows:

1. We used boxplot function on the age-length data to remove outliers by year;
2. We used the age-length data without outliers to make annual ALKs from 2008 to 2019;
3. We used each annual ALK to convert its corresponding length distribution to age distribution;
4. There were three sets of converted age distributions as follows:
   1) Age distribution from the length distribution with the fleet with the largest sample sizes;
   2) Age distribution from the coast-wide length data from all sources, commercial, recreational, and all gears;
   3) The 2) age distribution but with the most younger ages removed.

   The purpose to examine the three age distributions is to see which one would provide the most information on cohort progressions through years.

Comparison between the age distributions and abundance indices

We compared the strong cohorts identified by age distributions and abundance indices, expecting that the age distributions may verify the stock abundance through years identified by the abundance indices.

von Bertalanffy growth parameters

We assumed the age-specific natural mortality was constant through years, was the same between sexes, and between regions, therefore, we used the region-, year- and sex-pooled age-length data collected between 1983 and 2020 (the terminal year for 2022 stock assessment). We fitted von Bertalanffy growth model $L_t = L_\infty[1 - e^{-K(t-t_0)}]$ using nonlinear least square function (assuming additive error structure) to the data to estimate the growth parameters, L_∞, K, and t_0. Before fitting the model to the data, we used boxplot function to remove outliers from the data by assuming that the length is normally distributed at each age. We fitted the model to both the mean length- and individual length-at-age data, respectively, in order to find which model is more appropriate to describe the black drum growth. The estimates of L_∞, K, and t_0 together with the Black Drum age range will be used to estimate age-specific natural mortality in the stock assessment (Lorenzen 1996; Then et al. 2015).

Results

Length data

Examination of length data

There were 2375 fish used to develop the linear model (Figure A1). This model was used to convert the folk length to total length for fish with folk length only. There are significant differences in mean length between gears within each state (Figure A2 and A3), and between states within each gear (Figure A4 and A5) except between FL gill net and FL hook and line (Top panel in Figure A3). Even though the lengths are significantly different between the majority of gears and all the states,

in order to increase sample sizes we made several fleets (Table A1) for further analysis (Please see detailed analysis in Jeff's working paper).

Length distributions to be converted

From Table A1 we picked NC commercial length data from 2008 to 2019 as the first length distribution (Figure A6) to convert it to its age distribution. Then, we used all the length data collected by both commercial and recreational using a variety of gears to make the second length distribution (Figure A7) for age conversion.

Age-length data and ALKs

In general, the sample sizes of age-length data from each state are very small and even the coast-wide sample sizes are very small before 2008 (Table A2), therefore, we didn't use any age-length data collected before 2008. Black Drum growth rates are significantly different between all the paired states (Not showing figures here), and we believe that such differences are mainly resulted from small sample sizes. However, there is no significant difference in growth between male and female Black Drum when all years and states data are pooled (Figure A8 and A9). There is no significant difference in growth between Mid- and South Atlantic region (Figure A10 and A11). Based on the results, we collapsed sexes and states within each year to make an annual ALK. Figure A12 shows the age-length data we used to make the annual ALKs and Figure A13 shows the age distribution in each ALK.

Converted age distributions

NC age distribution

Since NC gill nets collected mainly small fish (the majority $<$ 24 inch) (Figure A6), its age distributions are mainly young fish (the majority younger than Age 4) (Figure A14). As a result, NC age distribution is not able to provide any information on cohort progressions through years.

Coastal wide age distribution

The coast-wide length data did include more large fish, however, no cohort progression can be tracked through years in the age distributions from 2008 to 2019 mainly because the abundances of Age 3 and younger are significantly higher than the fish older than Age 3 (Figure A15).

Coastal wide partial age distribution

After removing fish Age 3 and younger, we are able to track four strong cohort progressions (2001, 2005, 2007, 20011) through years (Figure A16). Some strong cohorts are tracked more easily than others, for example, Year-class 2001 can be tracked through 11 of 12 years (lost tracking in 2016). Year-class 2015 is identified as a strong cohort, we may be able to track its progression through years after collecting more age-length and length data in the coming years.

Comparison between the age distributions and abundance indices

The strong cohorts identified by the age distributions do match those identified by abundance indices provided by NC (Figure A17) and SC (Figure A18).

von Bertalanffy growth parameters

There were 9378 samples of black drum collected between 1983 and 2020 with both age and length, of which 221 samples were identified as outliers, and 9157 samples were kept for further analysis (Figure A19). Figure A20 and A21 show the von Bertalanffy growth curves estimated using the mean length- and individual length-at-age, respectively. The predicted length at Age 0 is 328 and 242 mm from the mean length- and individual length-at-age model, respectively. Based on the observed length data for Age 0 Black Drum, we believe that the model developed from the individual length-at-age data is more appropriate to describe the Black Drum growth rate. As a result, we will use L_∞ of 1156, K of 0.133, and t_0 of -1.77 (Figure A21) in the development of age-specific natural mortality.

Discussion

This study used the observed length distribution (or raw length distribution) instead of the converted length distribution (or expanded length distribution) to track cohort progressions through years, providing three advantages as follows:

1. Catch in number is not required, as a result, no need to figure out how many catch is from which gear and how many fish should be converted from a catch in weight;

2. Since we are only interested in if the raw length can provide any information on cohort progression, we may collapse all the gears together because the gear selectivity will not influence our analysis as long as we have as a large sample size as possible and cover as a wide length range as possible;

3. When converting a length distribution to its age distribution, very often the length intervals in an ALK may not completely match those in the corresponding length distribution due to small sample sizes of and a wide range of Black Drum length. For example, an ALK lacks 10" interval whereas a length distribution lacks 11" interval. In this study we can delete the 10" interval from the ALK and the 11" interval from the length distribution, making the rest intervals completely match between the two. when an expanded length distribution is used, removal of any length intervals from the length distribution will underestimate the total catch in the CAA because the fish in the removed length intervals will not contribute to the CAA. To overcome such a loss of fish, people may pool two or more intervals together, which could result in pooling different cohorts together, reducing the CAA's ability to track cohort progression.

The results from this study are limited to tracking cohort progression through years, and may help identify which abundance index may be used in stock assessment. The method in this study may not be used to generate any CAAs since gear selectivity influences size of fish in catch and different states harvest different length ranges, as a result, pooling different gears and states may mistakenly distribute fish in catch into wrong length intervals.

We fitted the von Bertalanffy growth model to both mean length- and individual length-at-age. The mean-length method estimated a higher L_∞ and a lower K whereas the individual-length method estimated a lower L_∞ and a higher K, demonstrating an intrinsic inverse relationship between L_∞ and K (Quinn and Deriso 1999). Based on the values of L_∞ and K alone, we were unable to decide which method was more appropriate. However, there are two reasons for which we believe the individual-length method is more appropriate as follows:

1. The t_0 of -1.77 from the individual-length method is much closer to 0 than the t_0 of -3.28 from the mean-length method;
2. The predicted length at Age-0 from the individual-length method (242 mm) is much closer to the observed mean length at Age-0 than the one from mean-length method (328 mm).

Therefore, we believe that the individual-length method had a better fit, and its estimates of growth parameters are more representative of the Black Drum population growth.

The t_0 value closer to 0 in the individual-length method is most likely due to the significant large sample size of Age-0, in other words, it is a sample size effect. A simple way to get rid of a sample size effect is to fit the model to mean length-at-age data. However, in this case the mean-length method doesn't have a better fit and doesn't provide a more realistic estimate of length for Age-0 fish. As a result, we will use the parameters from the individual-length method for natural mortality estimation.

Goodyear (2019) discussed the influence of biased estimates of L_∞ and K on natural mortality estimate (M). The L_∞ and K of the individual-length method may not be free of biases even though the method seems having a better fit and providing a more realistic estimate of length at Age-0. A better fitting and a closer estimate of length to the observed mean length at Age-0 could simply describe the data better, and may not necessarily describe the population growth better when the age-length data are not representative of the population (Goodyear 2019). Therefore, we suggest that more effort should focus on improvement of age-length collection along Atlantic coast.

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Table A1: Sample sizes of the length data collected from commercial fisheries by fleet and year.

Year	DE North Gill Nets	MDVA Gill Nets	MDVA Fixed	MDVA Hook&Line	NC Ocean Gill Nets	NC Estuarine Gill Nets	NC Long Haul/ Trawls/Fixed	South All Gears
1989	0	25	12	0			0	11
1990	0	4	35	0			0	9
1991	0	87	22	0			0	50
1992	0	39	0	0			0	39
1993	0	11	84	0			0	57
1994	0	129	5	0	0	26	19	86
1995	0	1	5	0	17	2	145	31
1996	0	28	35	0	1	18	182	49
1997	0	203	7	0	1	24	65	40
1998	0	77	18	1	0	27	44	93
1999	0	201	10		2	114	472	177
2000	0	110	12	0	7	240	516	138
2001	0	104	46	5	4	166	243	176
2002	0	39	35	17	0	579	1254	77
2003	0	4	25	0	35	349	193	96
2004	0	0	73	0	2	269	94	79
2005	0	11	14	0	17	377	84	68
2006	0	3	14	0	18	1052	783	70
2007	0	3	15	0	17	1540	346	112
2008	0	0	14	0	57	1915	1016	174
2009	63	1	39	0	28	984	126	141
2010	84	23	14	1	2	469	190	136
2011	59	0	5	0	233	932	216	83
2012	23	20	16	0	14	1185	254	63
2013	45	26	48	0	50	989	174	97
2014	58	7	39	0	1	692	60	103
2015	90	0	20	0	4	469	99	71
2016	0	392	59	0	3	791	297	61
2017	63	0	48	28	10	1087	80	63
2018	86	74	49	57	3	469	196	61
2019	6	2	46	16	0	287	248	61
2020	45	3	28	0			19	100

Table A2: Sample sizes of the age-length data collected from coast-wide, by region, state, and year.

Year	Coastwide	Mid-Atlantic	South Atlantic	NJ	DE	MD	VA	NC	SC	GA	FL
1981	0	0	0	0	0	0	0	0	0	0	0
1982	0	0	0	0	0	0	0	0	0	0	0
1983	22	0	22	0	0	0	0	0	0	0	22
1984	101	0	101	0	0	0	0	0	0	0	101
1985	27	0	27	0	0	0	0	0	1	0	26
1986	46	0	46	0	0	0	0	0	46	0	0
1987	73	0	73	0	0	0	0	0	73	0	0
1988	0	0	0	0	0	0	0	0	0	0	0
1989	0	0	0	0	0	0	0	0	0	0	0
1990	0	0	0	0	0	0	0	0	0	0	0
1991	26	0	26	0	0	0	0	0	26	0	0
1992	38	0	38	0	0	0	0	0	38	0	0
1993	87	0	87	0	0	0	0	0	87	0	0
1994	29	0	29	0	0	0	0	0	29	0	0
1995	16	0	16	0	0	0	0	0	16	0	0
1996	52	0	52	0	0	0	0	0	52	0	0
1997	66	0	66	0	0	0	0	0	66	0	0
1998	83	6	77	0	0	0	6	0	46	31	0
1999	141	80	61	0	0	0	80	0	42	19	0
2000	182	42	140	0	0	0	42	0	113	27	0
2001	148	86	62	0	0	0	86	0	35	27	0
2002	242	70	172	0	0	0	59	0	135	37	0
2003	180	36	144	0	0	0	11	0	76	67	1
2004	68	18	50	0	0	0	14	0	29	21	0
2005	62	28	34	0	0	0	8	0	26	8	0
2006	51	15	36	0	0	0	7	0	27	9	0
2007	139	57	49	0	0	0	35	0	24	23	2
2008	409	206	176	0	26	0	171	0	10	166	0
2009	317	171	83	0	97	0	61	0	25	58	0
2010	394	211	172	0	129	0	71	0	19	153	0
2011	368	115	205	0	90	0	19	175	13	13	4
2012	458	55	387	0	33	0	19	307	11	45	24
2013	422	108	294	0	58	0	42	178	24	51	41
2014	670	178	468	0	62	0	102	393	7	47	21
2015	576	144	397	0	78	0	55	358	2	16	21
2016	1108	400	702	0	11	0	372	571	20	106	5
2017	812	153	618	0	59	0	63	562	31	20	5
2018	735	320	373	0	105	0	215	350	11	0	12
2019	558	139	419	0	47	0	92	375	19	0	25
2020	208	73	74	0	67	0	6	64	1	0	9

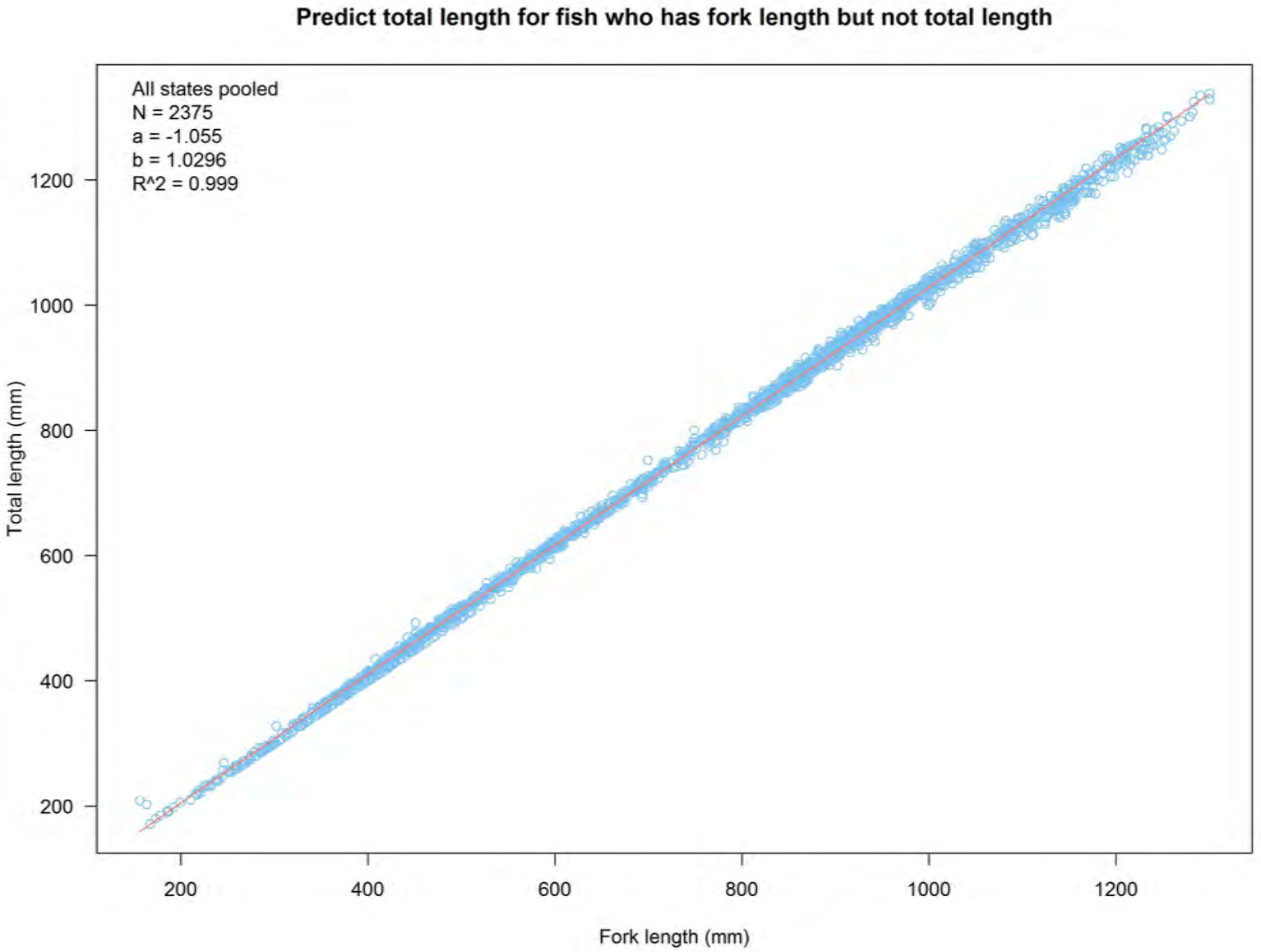


Figure A1: The relationship between fork and total length (mm) of Black Drum.

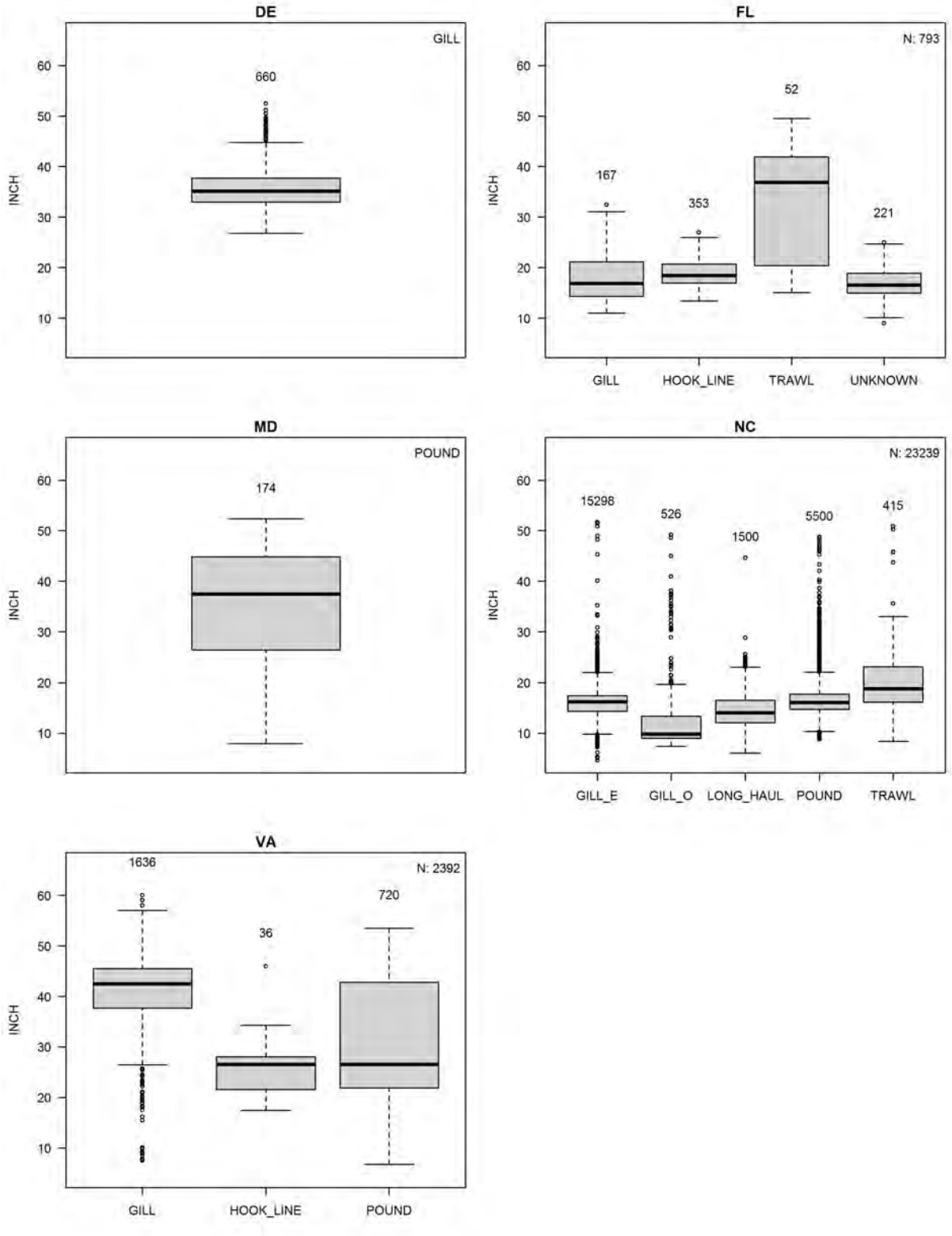


Figure A2: Comparison in the total length of Black Drum between gears within each state.

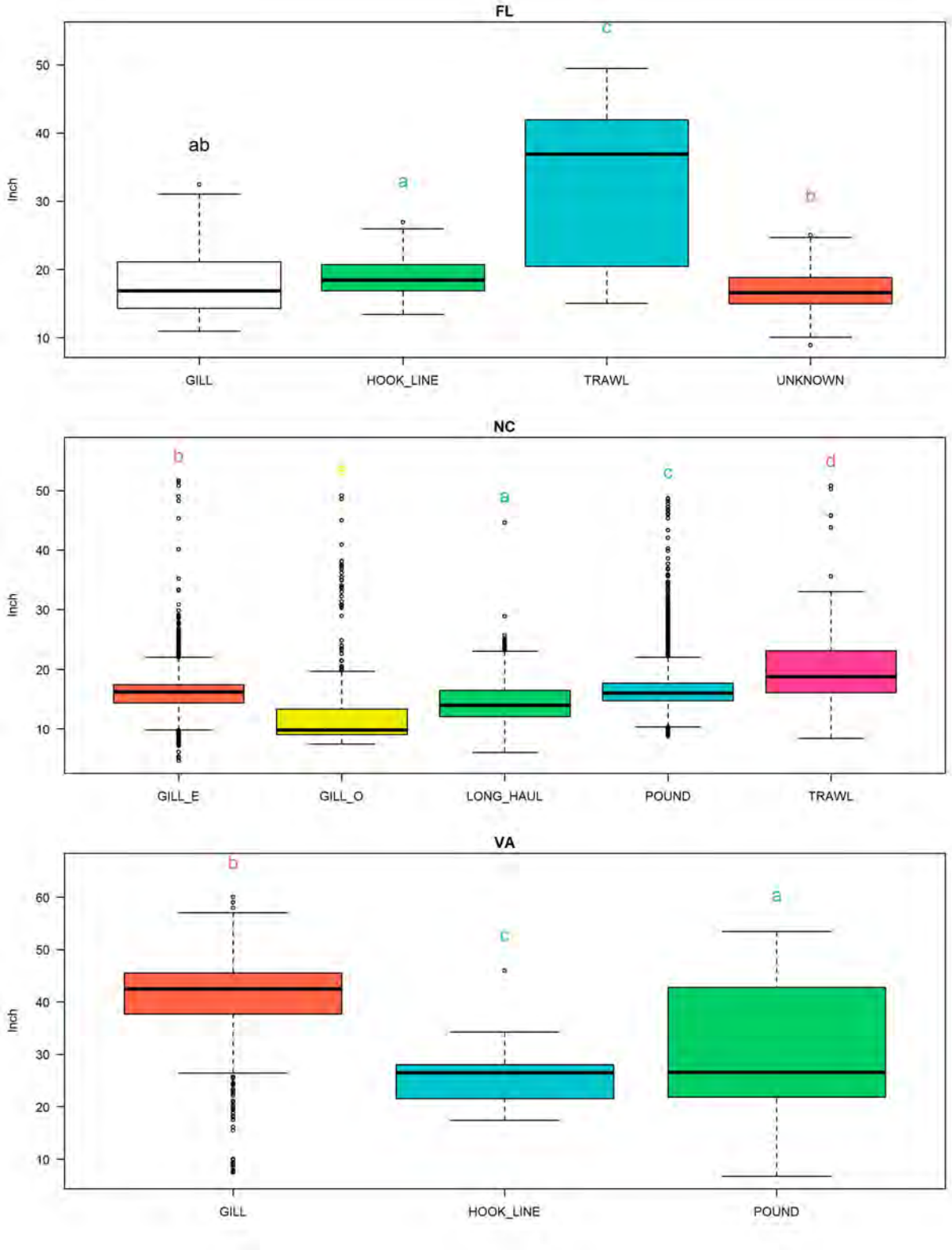


Figure A3: Tukey tests on the total length of Black Drum between gears within each state which has more than two gears. Two or more gears share the same letter are not significantly different.

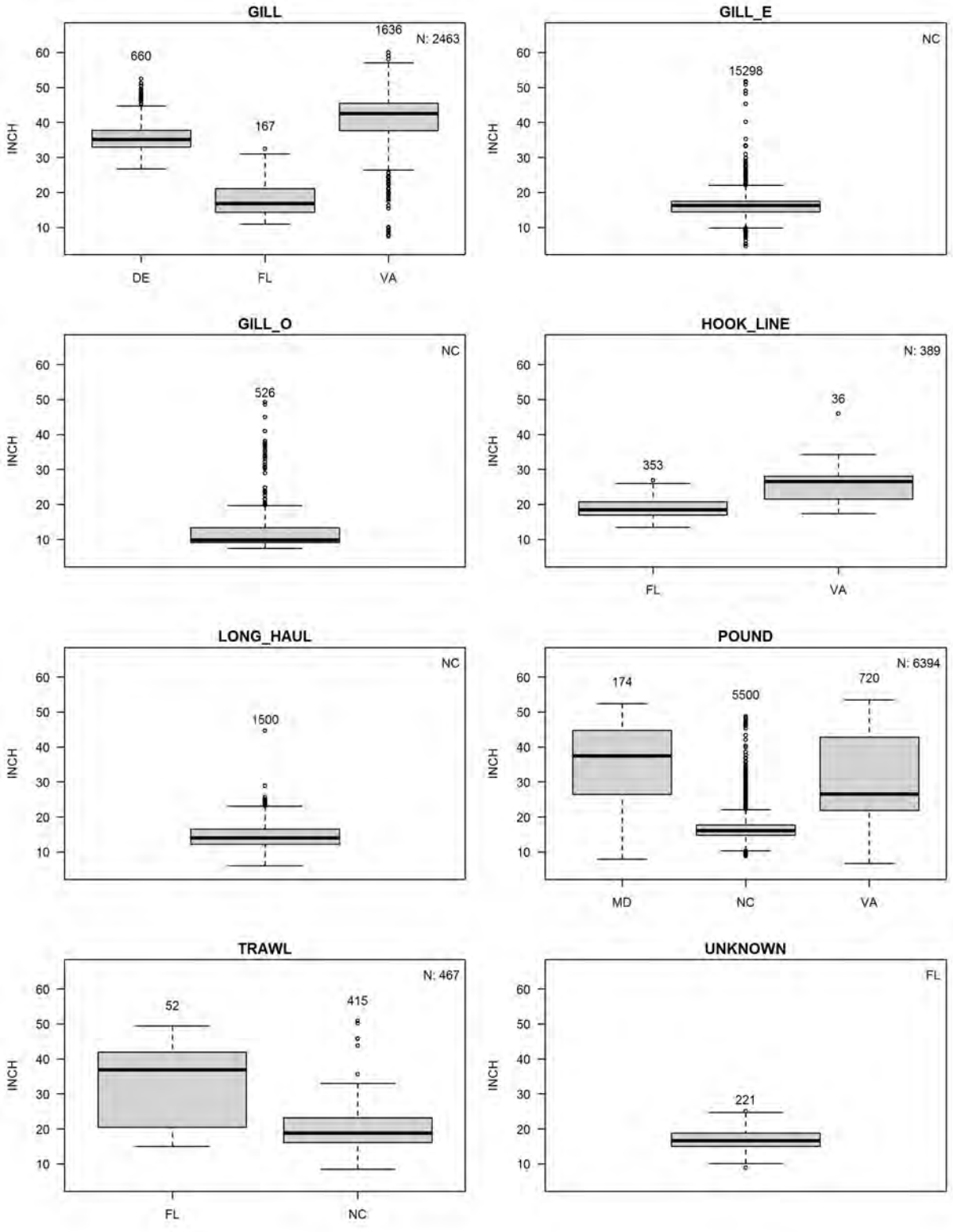


Figure A4: Comparison in the total length of Black Drum between states within each gear.

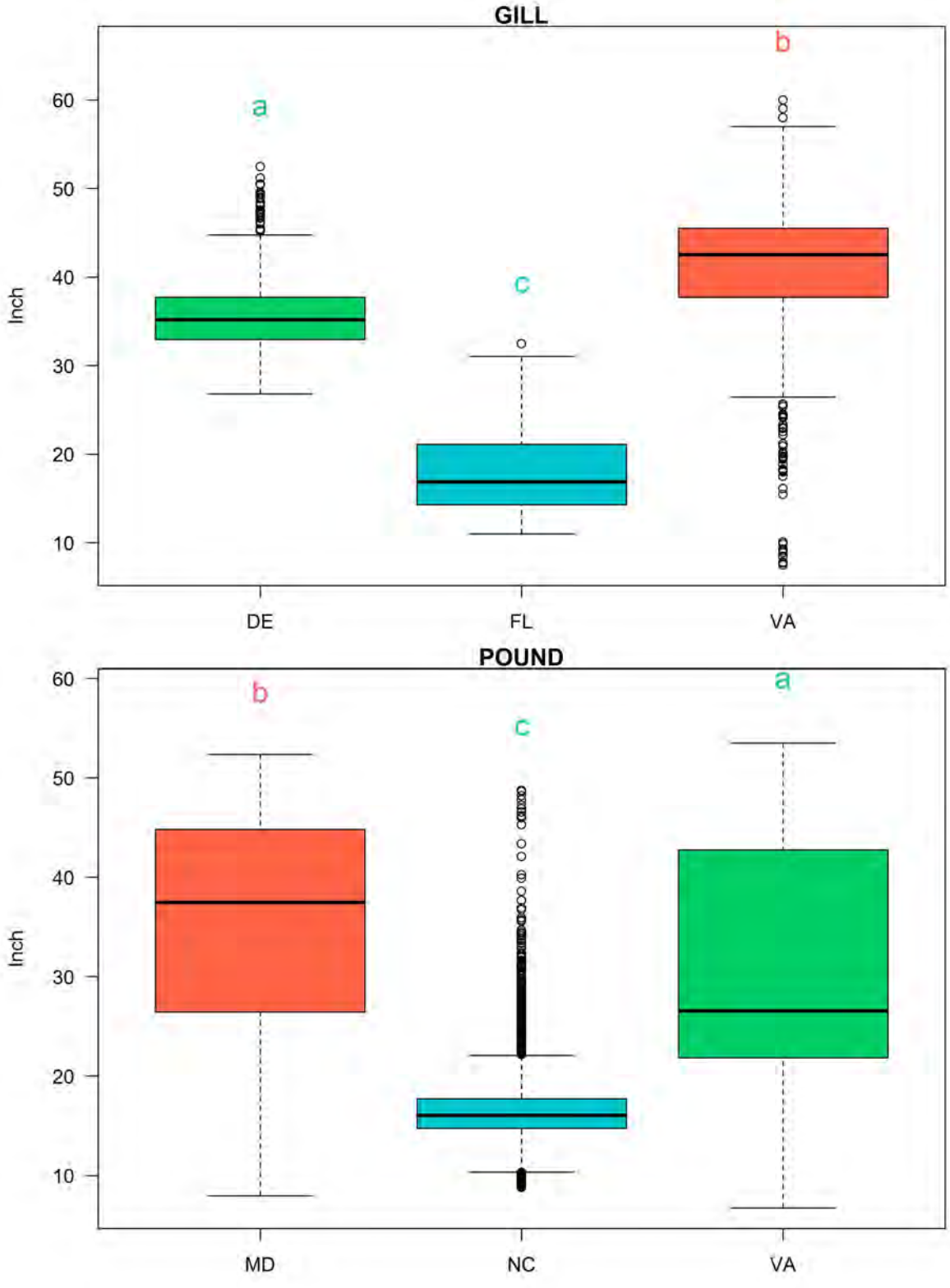


Figure A5: Tukey tests on the total length of Black Drum between states within each gear which has more than two states. Two or more states share the same letter are not significantly different.

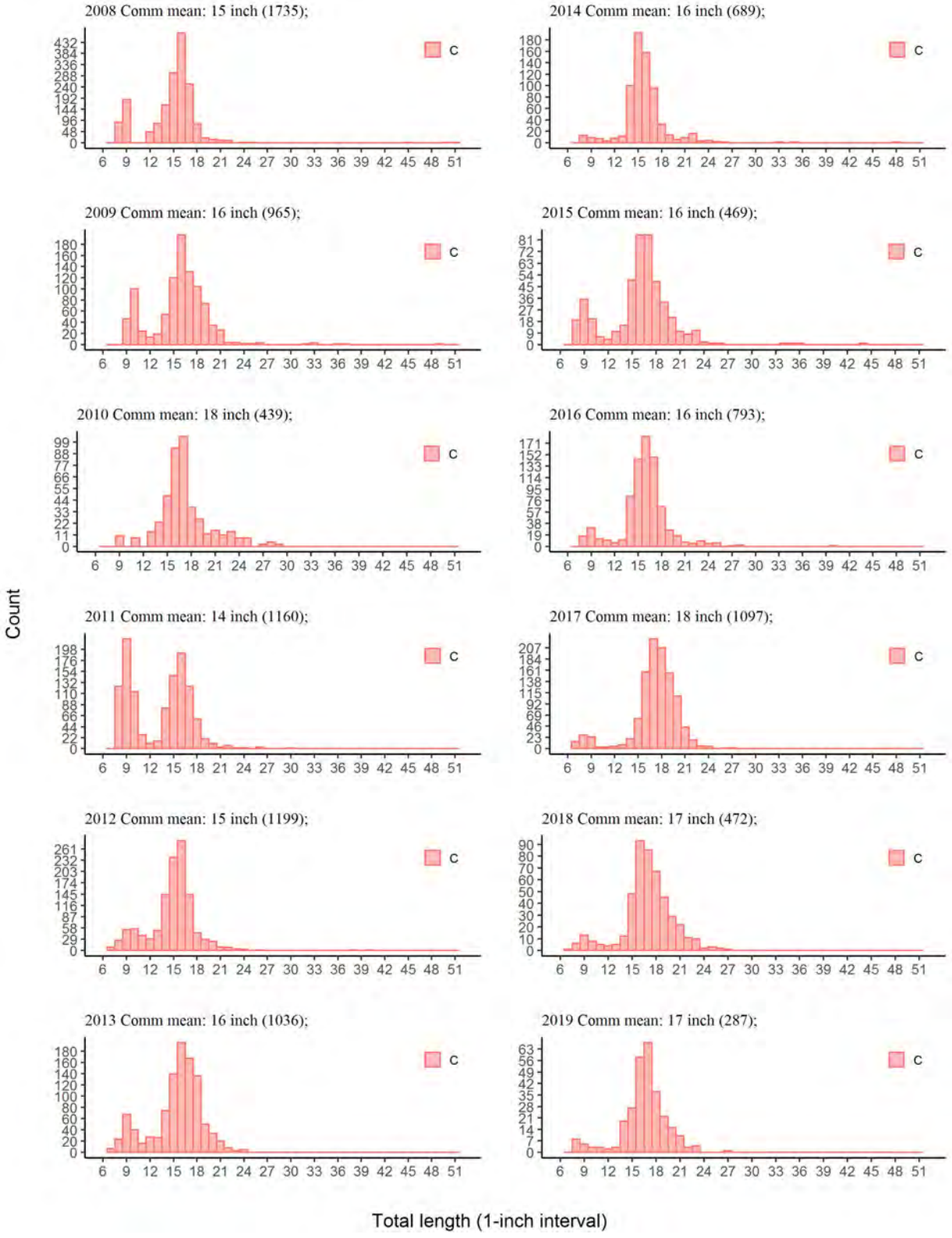


Figure A6: NC Black Drum length distribution (1-inch interval) collected from NC commercial fisheries from 2008 to 2019.

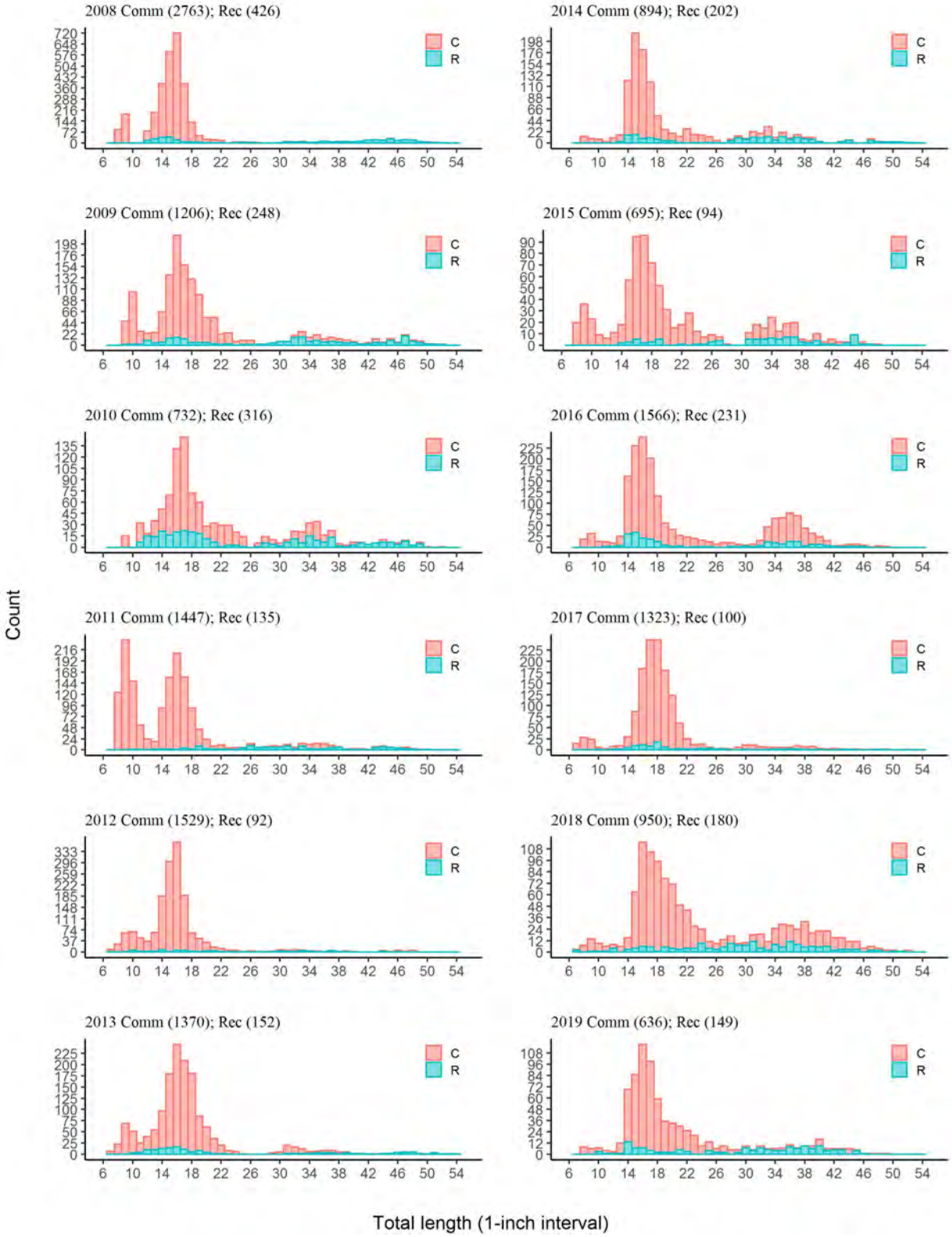


Figure A7: Coastal wide Black Drum length distribution (1-inch interval) collected from both commercial and recreational fisheries from 2008 and 2019.

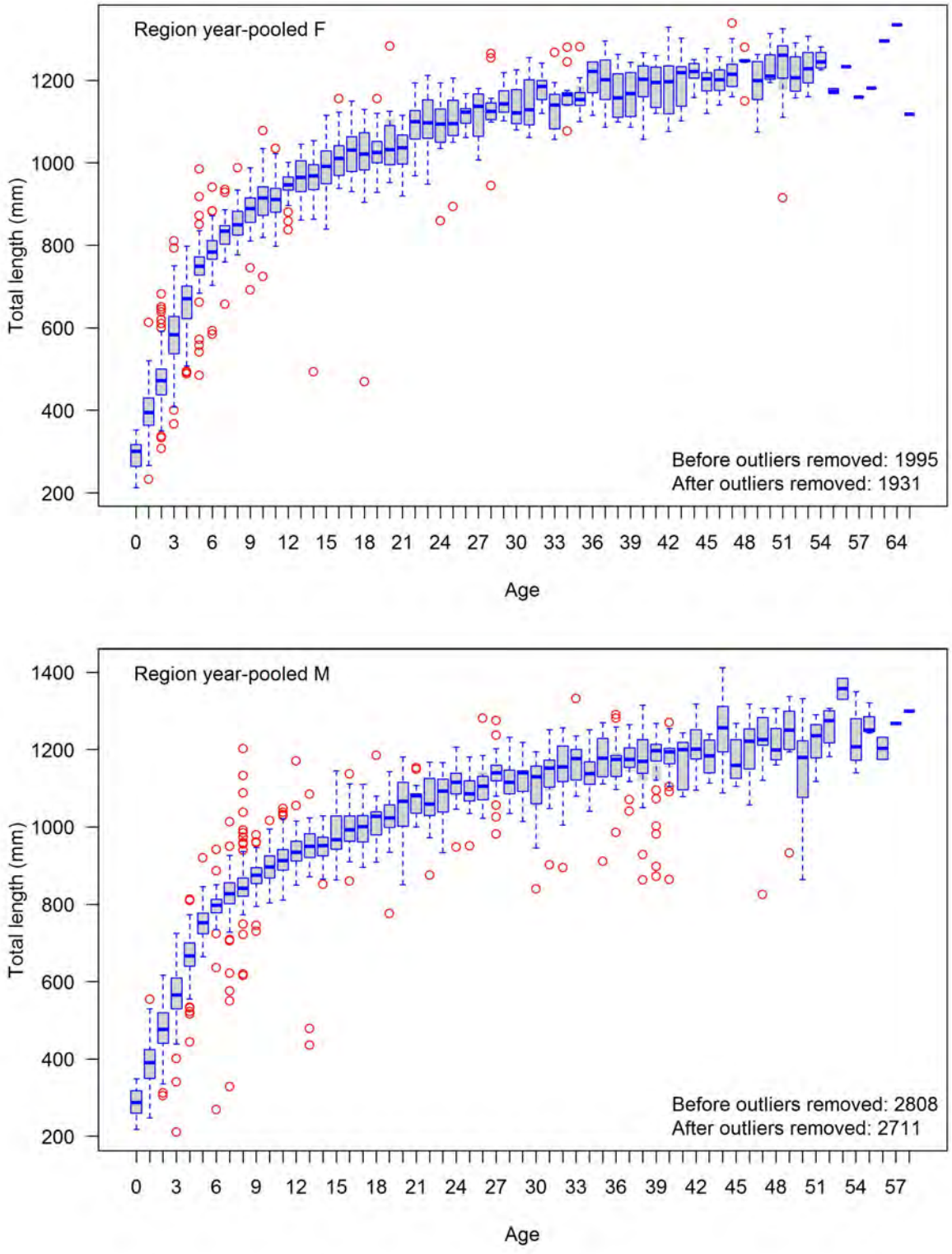


Figure A8: Age-length data before and after outlier removal by sex using boxplot function. "F" and "M" stand for female and male, respectively. One red circle represents one fish identified as an outlier.

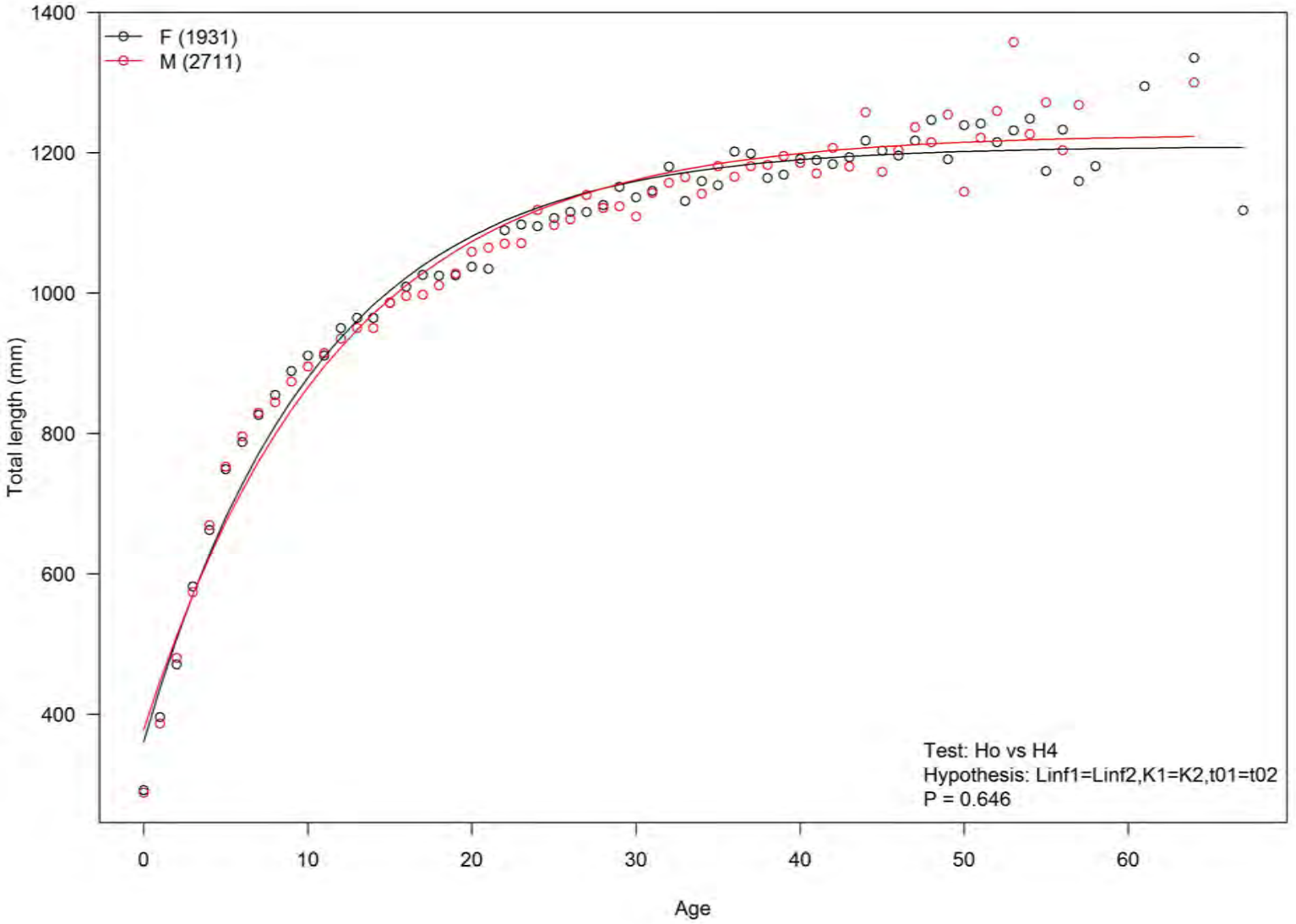


Figure A9: Kimura test on von Bertalanffy growth rates between coast wide and year-pooled female and male Black Drum. "F" and "M" stand for female and male, respectively. A data point is a mean length at age.

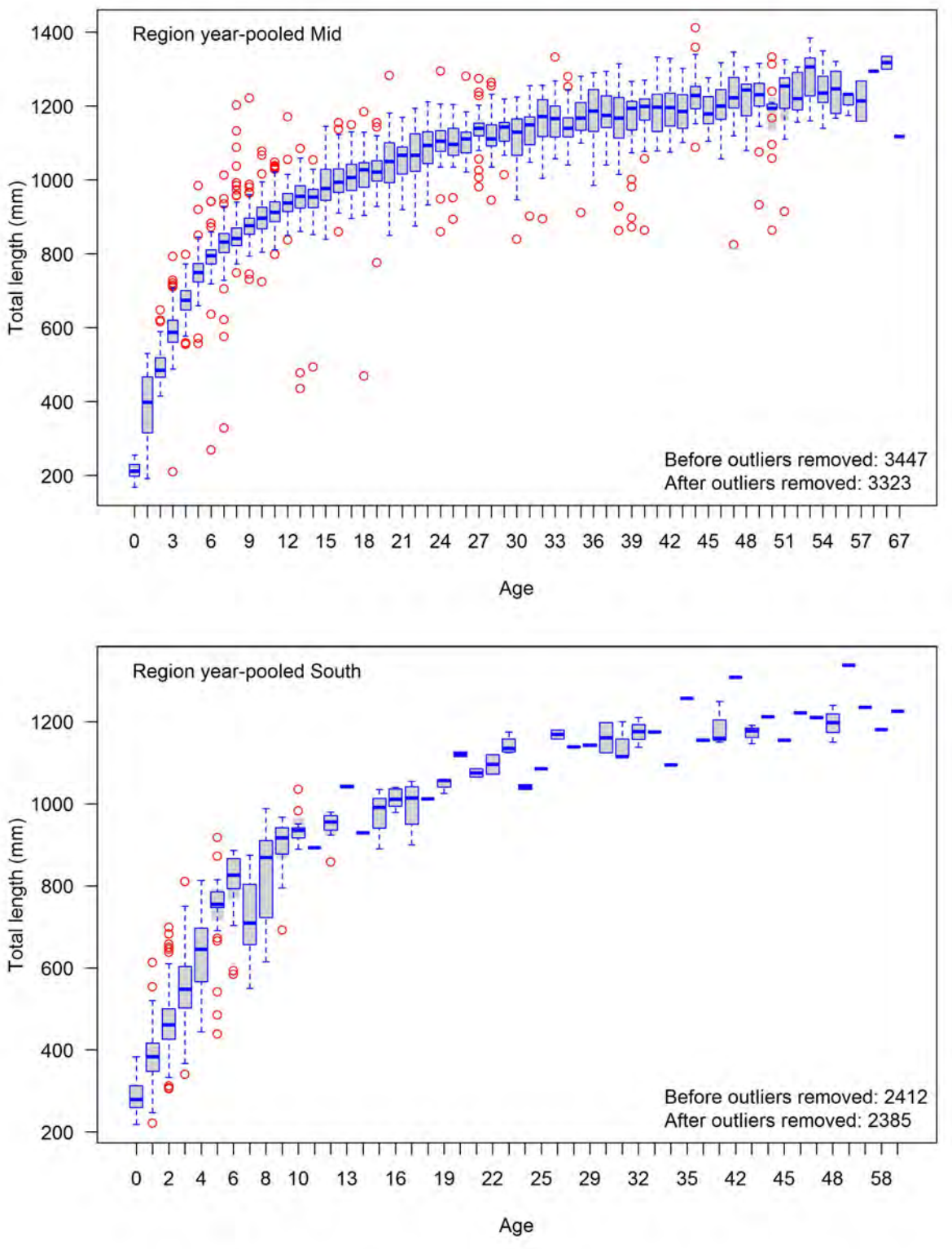


Figure A10: Age-length data before and after outlier removal by region using boxplot function. Mid-Atlantic includes NE, MD, and VA whereas South Atlantic includes NC, SC, GA, and FL. One red circle represents one fish identified as an outlier.

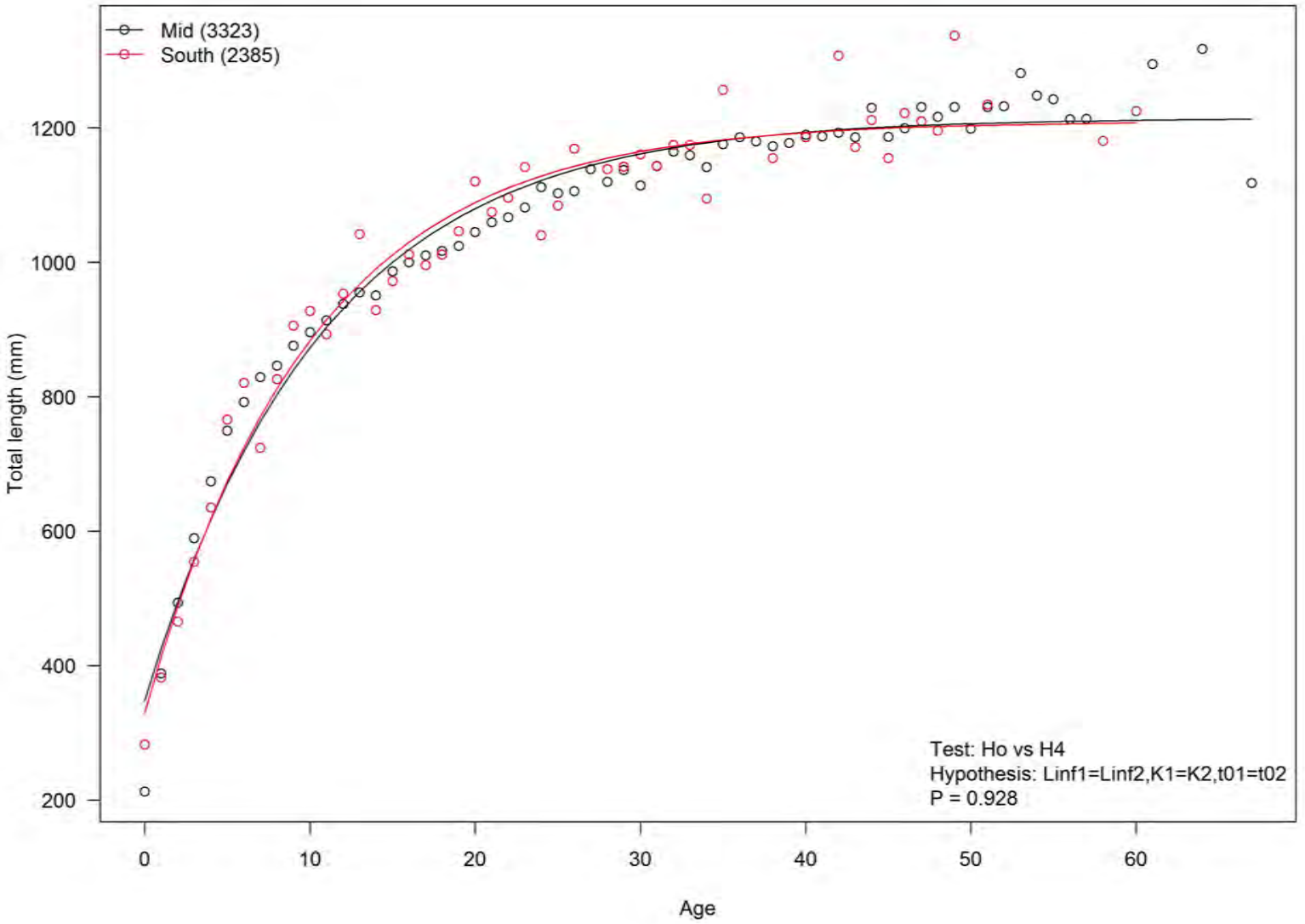


Figure A11: Kimura test on von Bertalanffy growth rates between coast wide and year-pooled Mid- and South Atlantic Black Drum. Mid-Atlantic includes NE, MD, and VA whereas South Atlantic includes NC, SC, GA, and FL. A data point is a mean length at age.

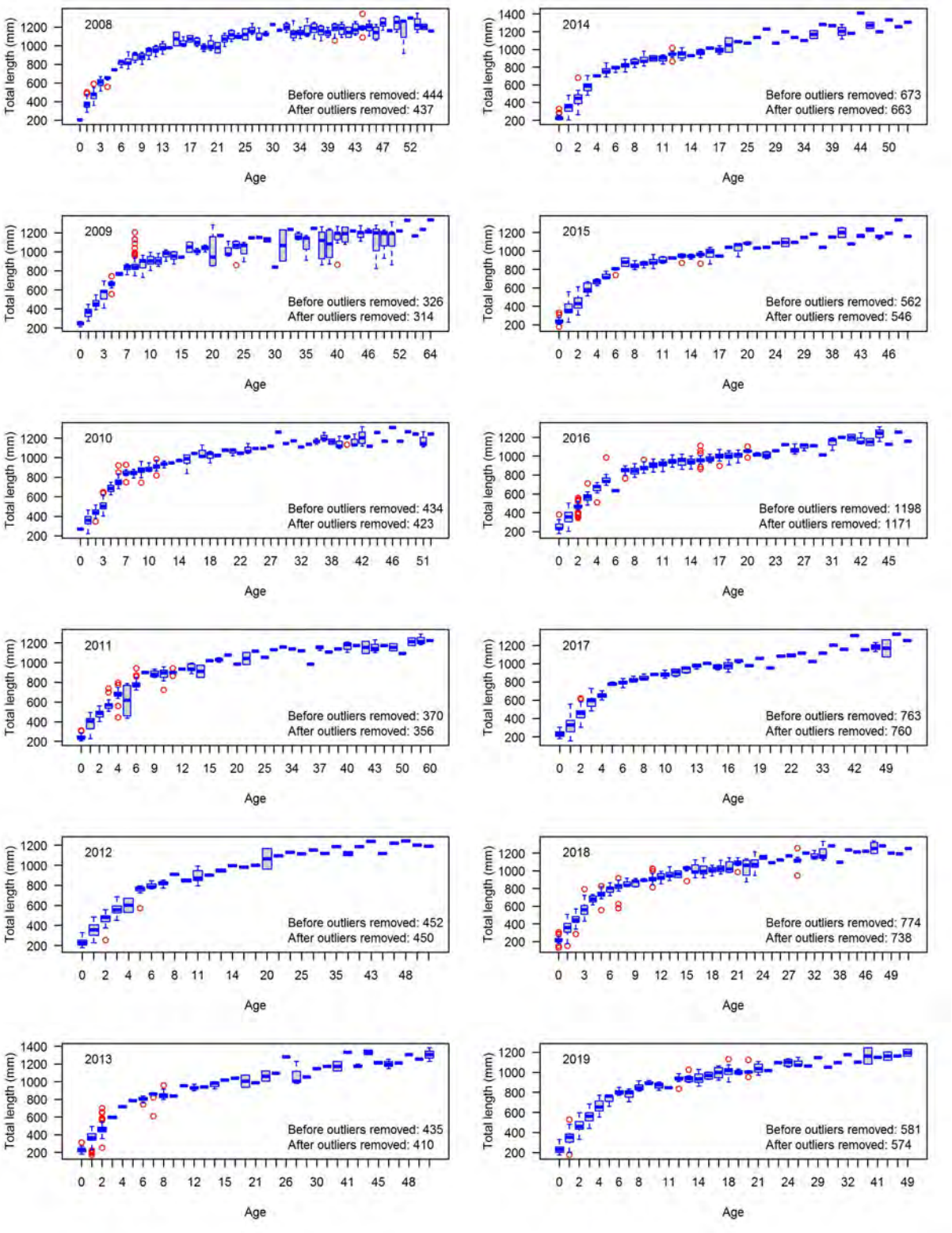


Figure A12: Coastal wide age-length data before and after outlier removal by year using boxplot function. One red circle represents one fish identified as an outlier.

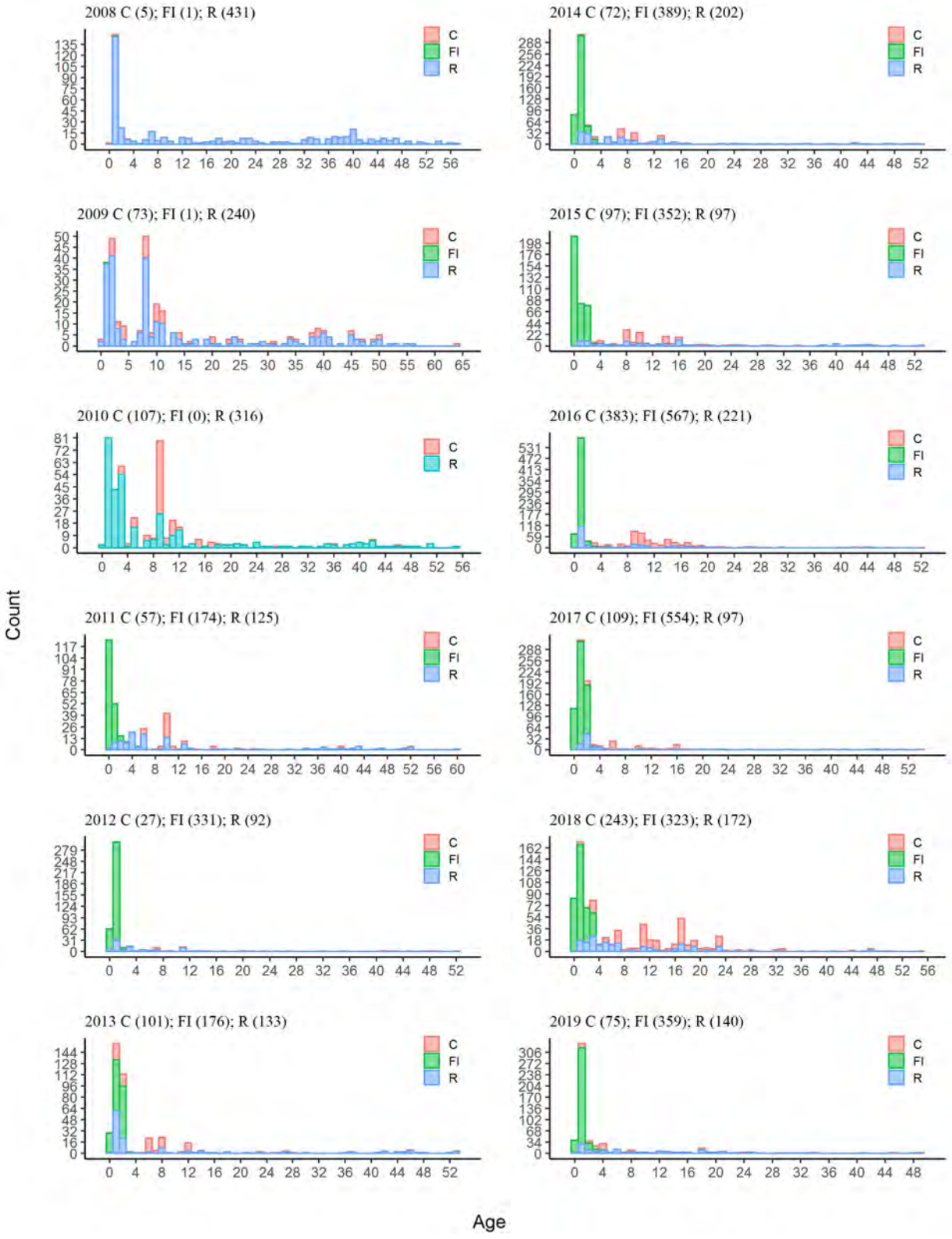


Figure A13: Coast-wide annual age distributions after outliers removed. "C", "FI", and "R" stand for the data collected from commercial fisheries, fishery independent survey, and recreational fisheries, respectively.

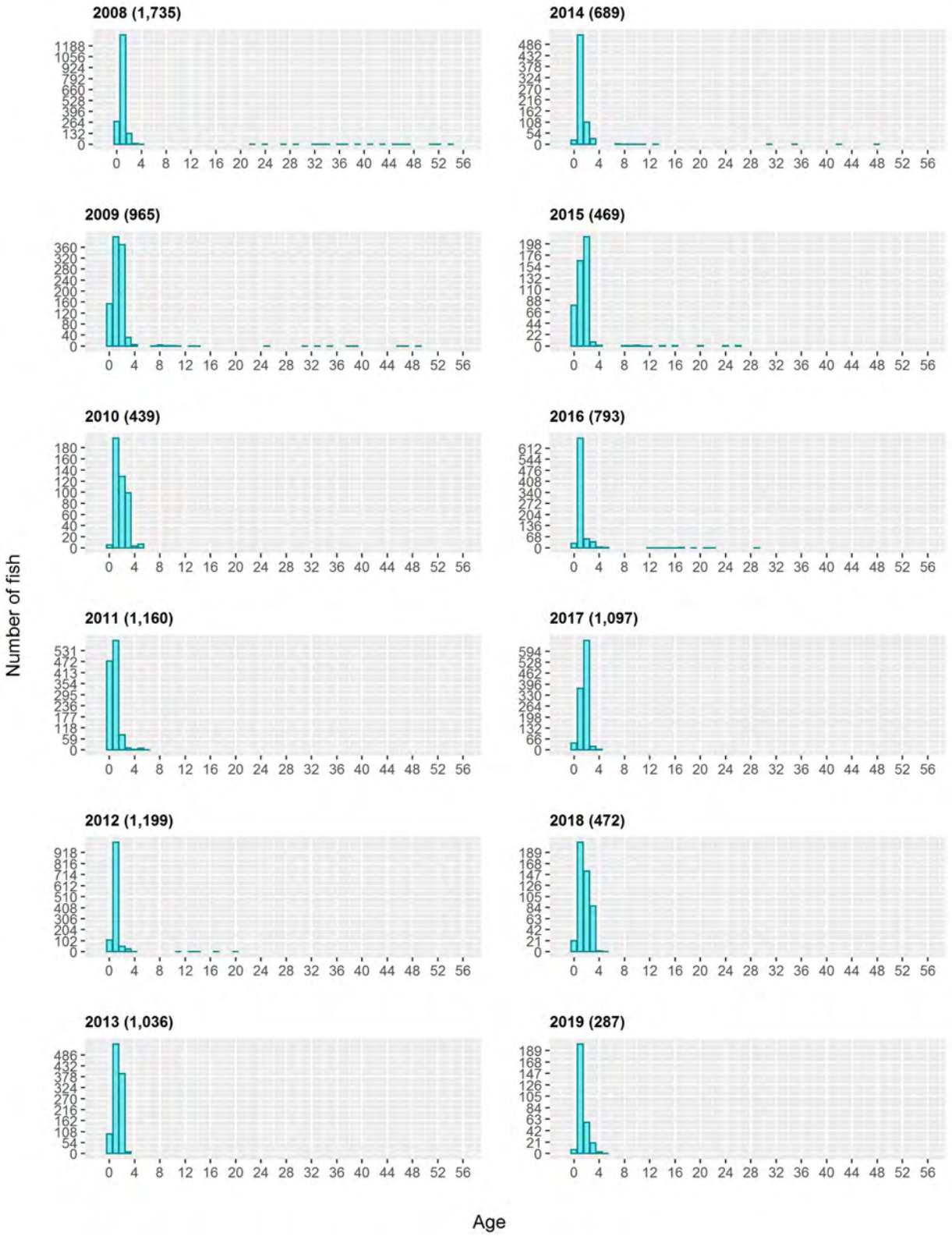


Figure A14: NC annual age distributions from 2008 to 2019 converted from NC annual length distributions using coast-wide annual ALKs.

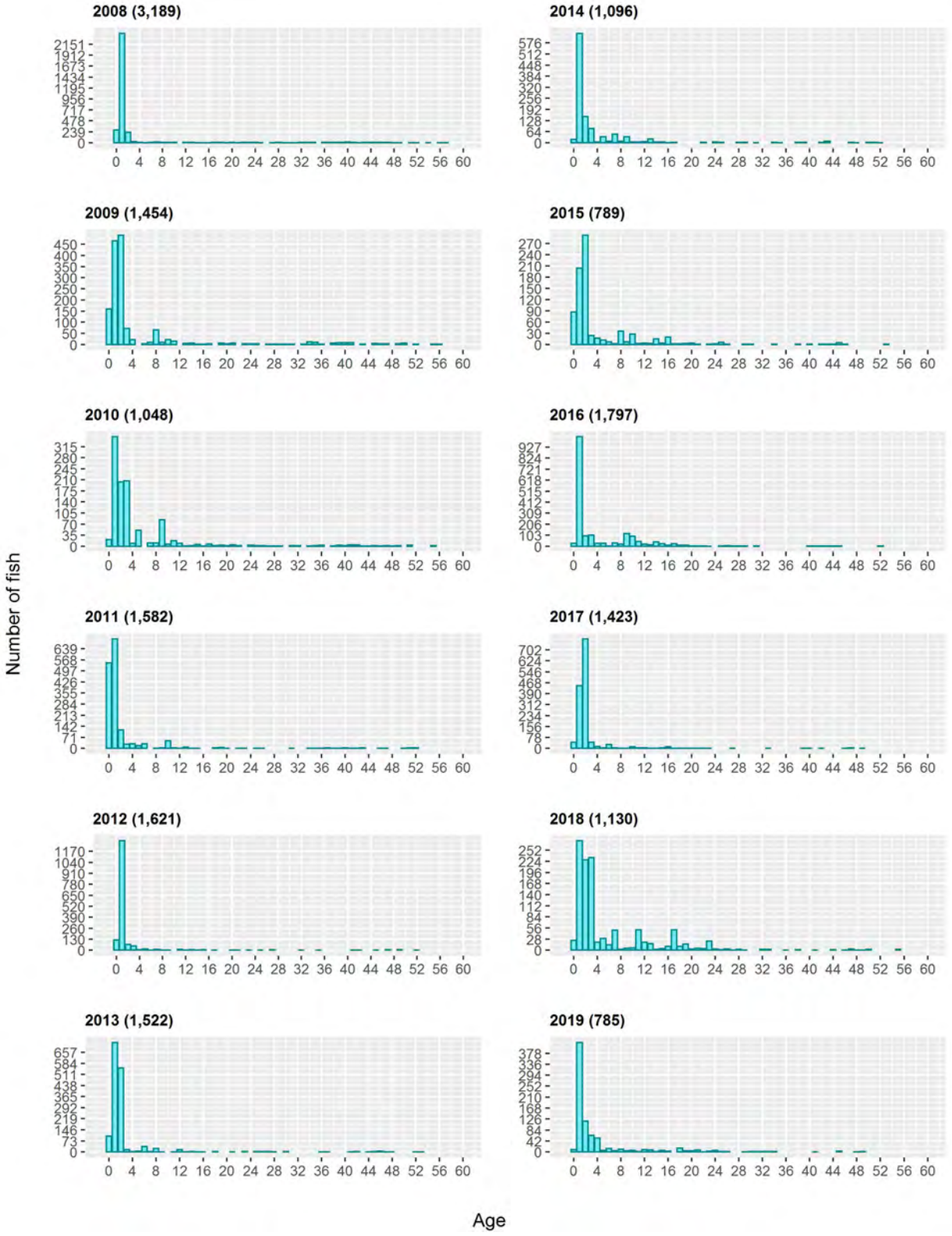


Figure A15: Coast-wide annual age distributions from 2008 to 2019 converted from coast-wide annual length distributions using coast-wide annual ALKs.

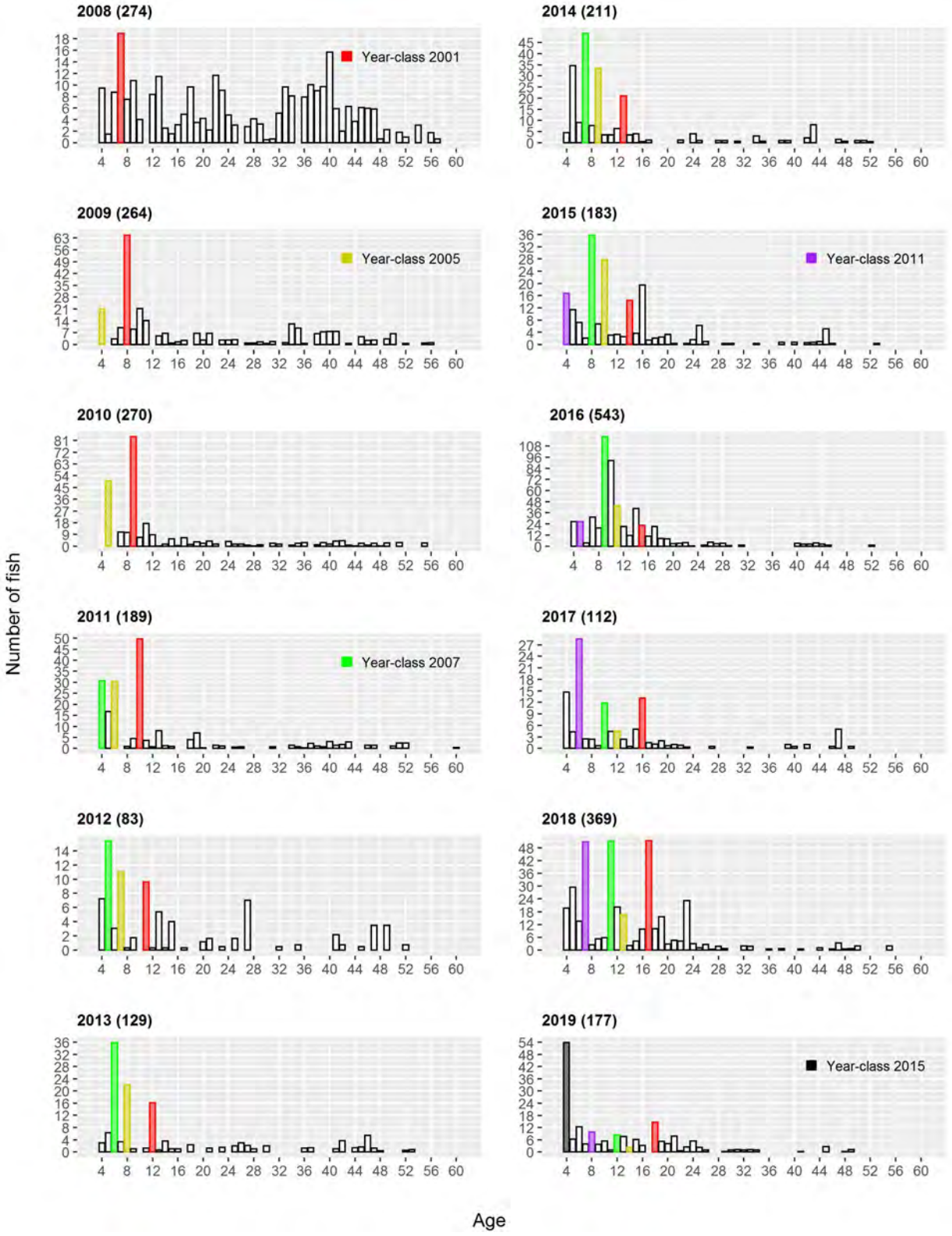


Figure A16: Coast-wide annual age distributions from 2008 to 2019 with removal of fish younger than Age 4.



Figure A17: NC abundance indices. X-axis is year-class.

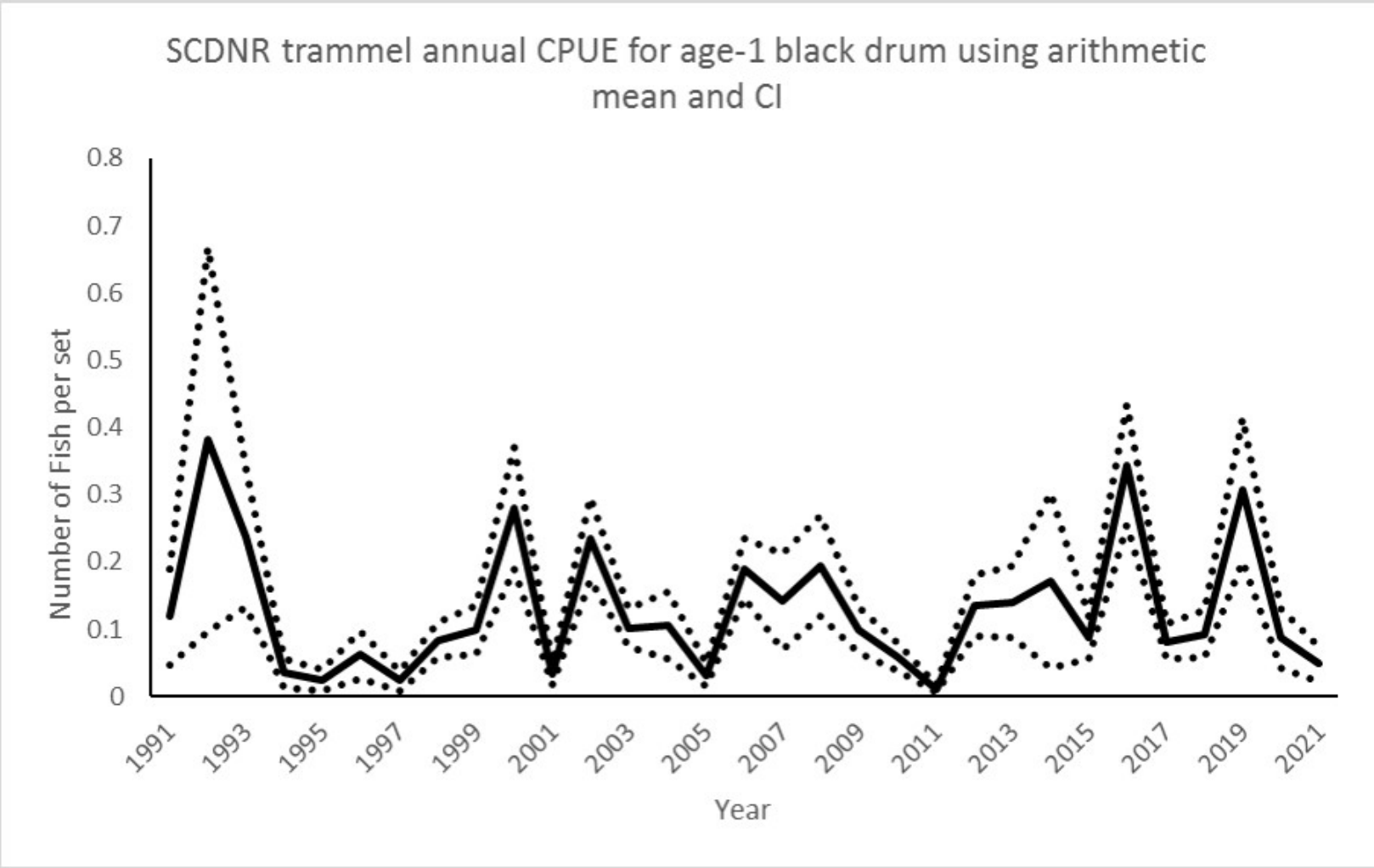


Figure A18: NC trammel net CPUE index for Age 1 of Black Drum.

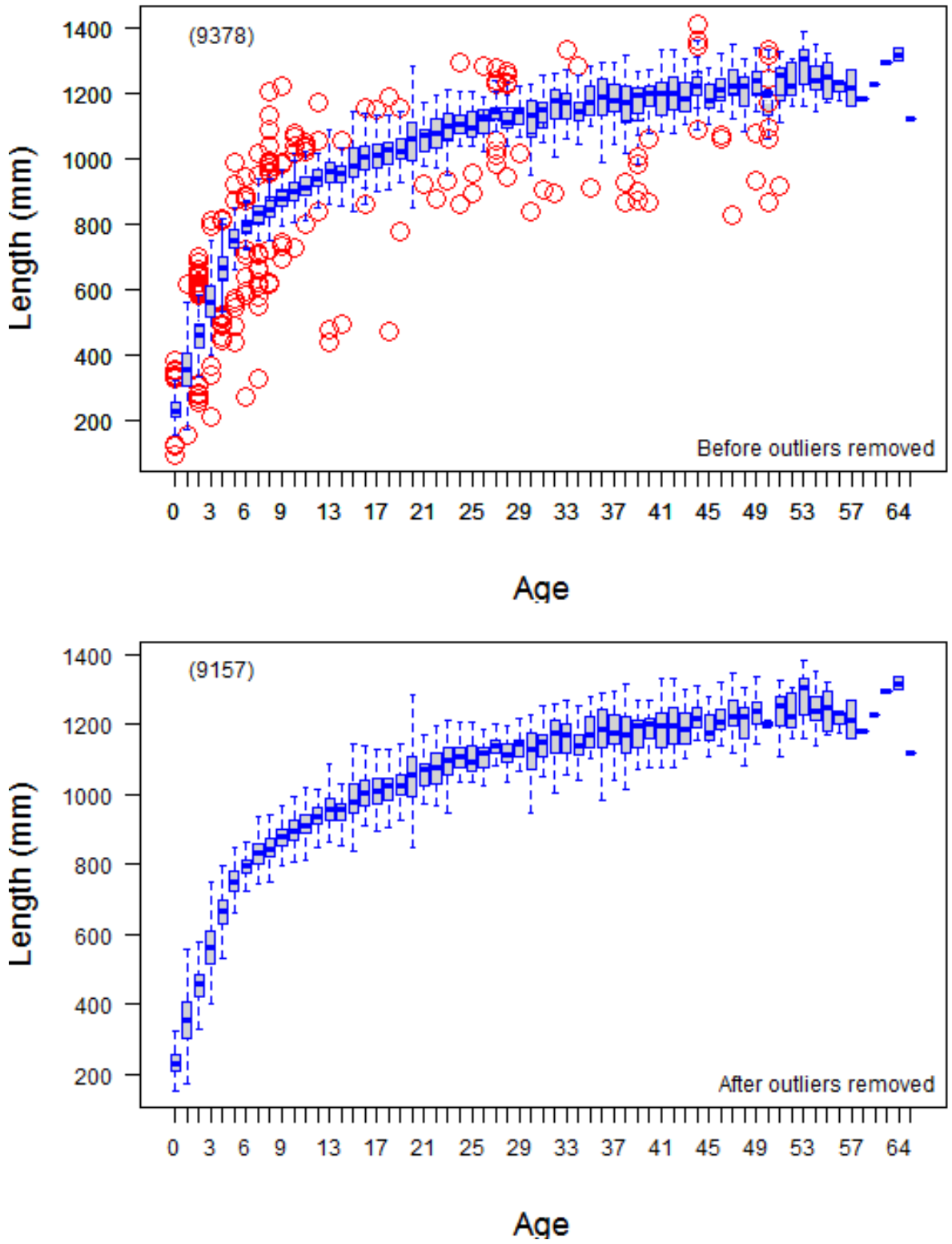


Figure A19: Outliers were moved from the coast-wide year- and sex-combined age-length data collected between 1983 and 2021 from recreational, commercial fisheries, and fishery-independent surveys. A red circle represents one fish identified as an outlier.

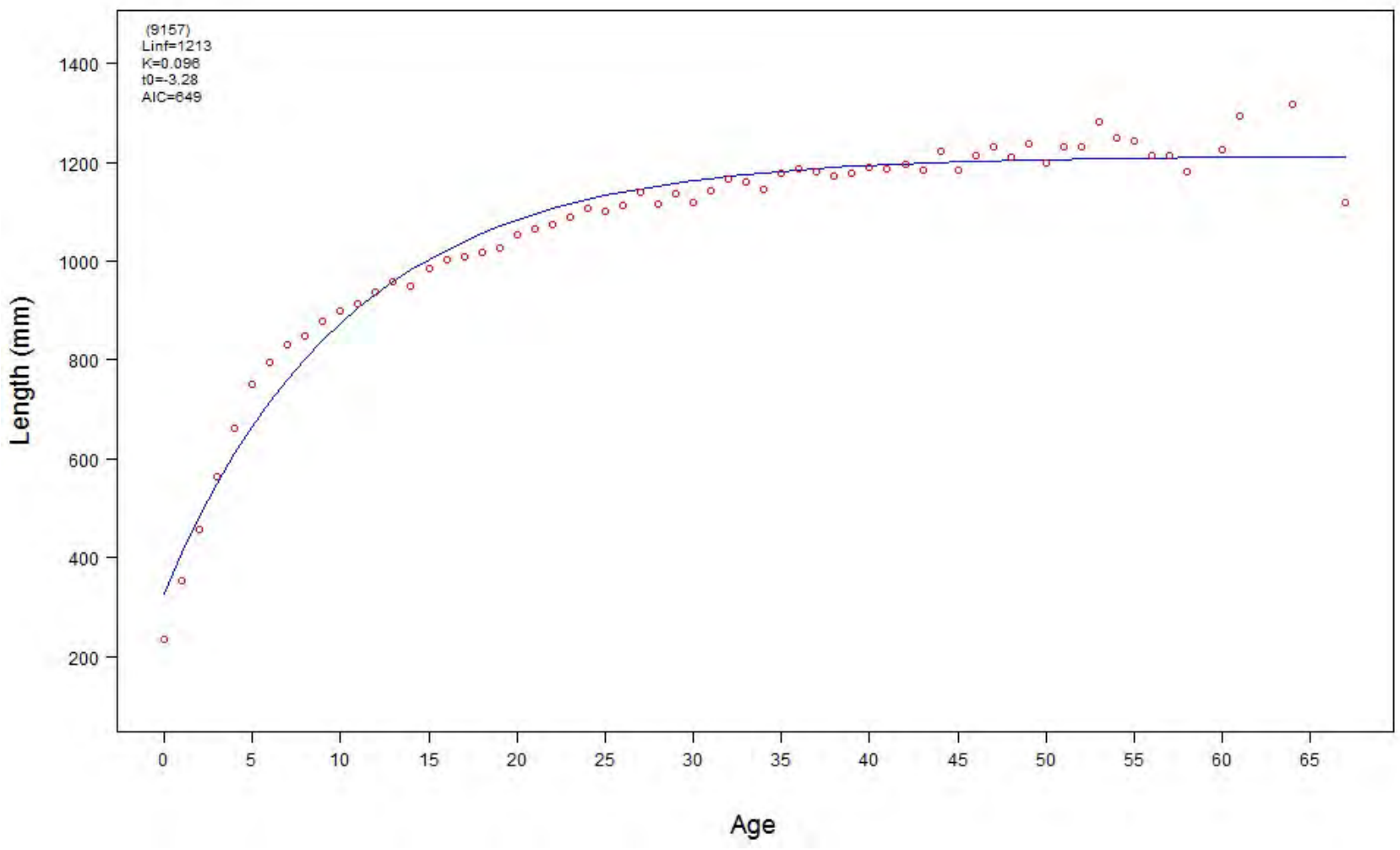


Figure A20: von Bertalanffy growth curve (blue line) with its parameters estimated using the region-, year-, and sex-pooled mean length-at-age data (red circles) collected between 1983 and 2020. The number in parenthesis is the sample size. The minimum age is 0 whereas the maximum age is 67.

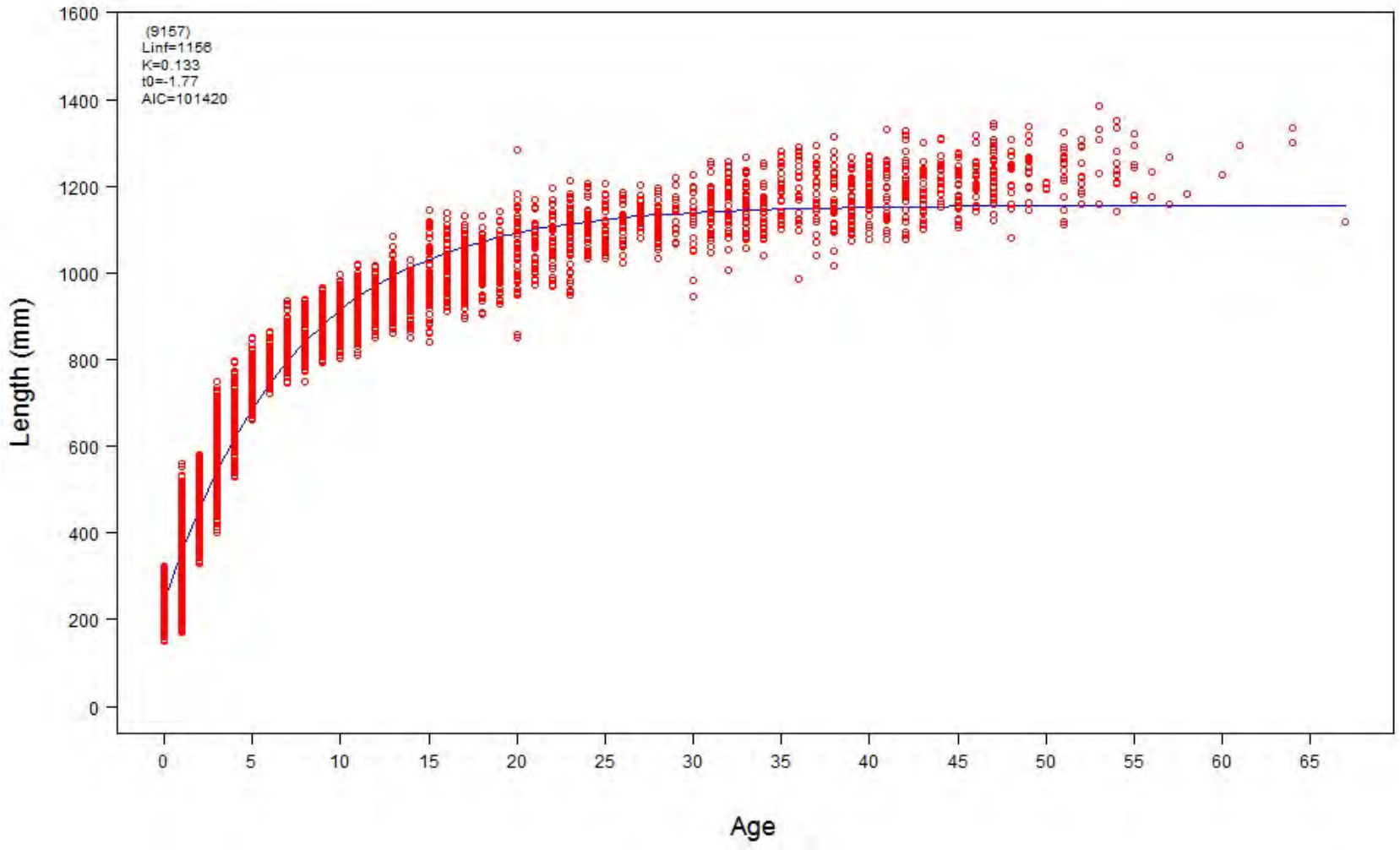


Figure A21: von Bertalanffy growth curve (blue line) with its parameters estimated using the region-, year-, and sex-pooled individual length-at-age data (red circles) collected between 1983 and 2020. The number in parenthesis is the sample size. The minimum age is 0 whereas the maximum age is 67.