Center for Quantitative Fisheries Ecology

Protocol

Age Estimation of Otolith Transverse Cross-Sections for

Spotted seatrout

Cynoscion nebulosus

Contents

Introduction

This protocol is for age estimation of spotted seatrout (*Cynoscion nebulosus*) using sagittal otolith transverse cross-sections. For detailed information on how to make a spotted seatrout otolith thin-section slide, please see the protocol for Preparation of Otolith Transverse Thin-sections for Age Estimation of spotted seatrout *Cynoscion nebulosus*. Hereafter, sagittal otoliths will be refered to as "otolith" and otolith transverse cross-section will be referred to as "thin-section".

General thin-section ageing procedure

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

Specific spotted seatrout ageing procedure

Ageing spotted seatrout otoliths involves two steps:

1. Reading the thin-section by counting the number of annuli.
2. Assigning an age to the fish based on sacrifice date and annulus formation period.

Step 1: Reading the thin-section:

1. Remove a labeled slide (Figure 1) with mounted spotted seatrout thin-section from the slide box labeled "Spotted seatrout, VMRC (Year)" (Figure 2).

Figure 1: Spotted seatrout thin-section mounted on labeled micro slide.

Figure 2: Otolith slide storage box labeled for spotted seatrout.

2. Place the slide on the microscope stage and turn on the transmitted light source. Adjust the dark-field polarization (Figure 3) until a dark blue background appears behind the otolith section. On other microscopes, the polarizer may be located on the base or stage of the microscope.
3. Adjust the coarse and fine foci until the entire otolith section is in clear view (Figure 4). A well-sectioned otolith will provide a clear view of the core, annuli, and sulcal groove. The annual rings will be plainly visible along its edge. If the section lacks a clear "V" shape within the sulcal groove, the cut did not go through the core. The otolith must be

Figure 3: Nikon SMZ 1000 stereo microscope with 1x objective and dark-field polarization.

re-sectioned following the otolith preparation protocol.

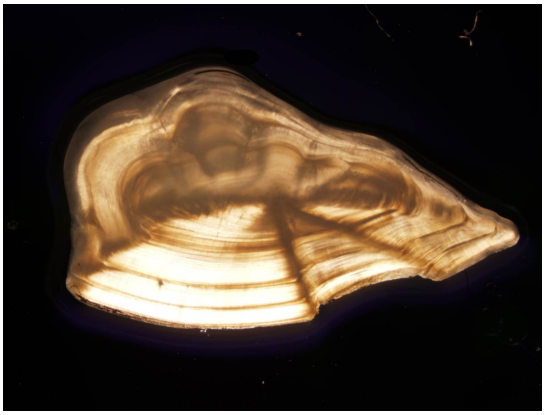


Figure 4: Spotted seatrout thin-section under transmitted light and dark-field polarization.

4. When a good section has been obtained, count each visible annulus on the section, starting from the core and moving to the proximal edge. Technically, an otolith annulus is a combination of opaque and translucent bands. When ageing otoliths however, only the opaque bands are counted. The translucent areas of the thin-section represent growth between annuli.

Step 2: Assigning an age to the fish

Once the number of annuli in the otolith, ("X"), has been identified, one of two scenarios determine the estimated age of the fish:

1. There is no growth beyond the last annulus: The age of the fish becomes an even "X". This typically happens when a fish has been collected during the annulus deposition period: March 1 to May 31 (Ihde and Chittenden 2003).

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

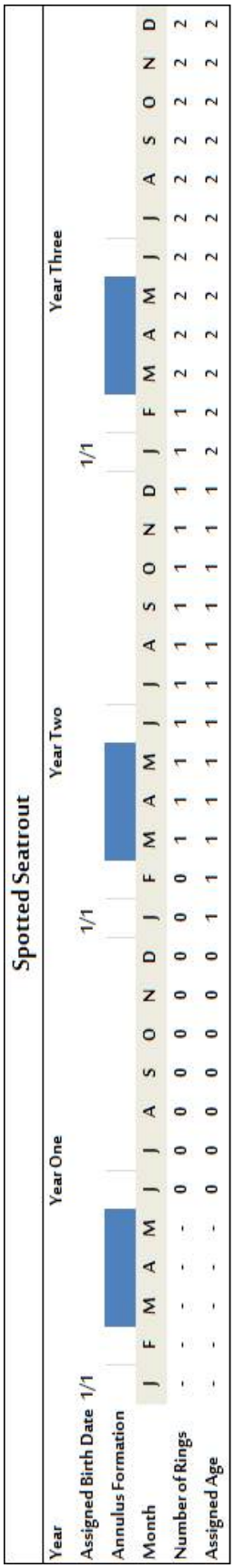
    2.1 If the sacrifice date for the fish is between January 1, the assigned birth date for all finfish of the Northern hemisphere, and the end of the last month in which spotted seatrout annuli are laid down, May 31, the age of the fish is represented as "X+(X+1)". For example, a fish with 2 visible annuli on its thin-section would be assigned the age "2+3", indicating that it belongs to the "age 3" age class.

    2.2 If the sacrifice date for the fish falls after May 31 and before January 1, the fish has laid down its annulus for the year and has experienced growth since that time. The age of the fish is represented as "X+(X)". A fish with 6 annuli visible on its otolith thin-section would be assigned the age "6+6", indicating that it belongs to the "age 6" age class.

Assigning age and year-class

The following images demonstrate how we assign the age and year-class to fish by using the age assigning table (Figure 5).

A spotted seatrout taken in February 2009 (before the annulus deposition period) with 1 visible annuli and translucent growth beyond the last annulus (Figure 6) would be



Spotted Seatrout																																			
Year					Year One												Year Two												Year Three								
Assigned Birth Date	1/1												1/1												1/1										
Annulus Formation																																			
Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Number of Rings	-	-	-	-	-	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2
Assigned Age	-	-	-	-	-	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2

Figure 5: Spotted seatrout age assigning table

called "1+2" and put into the 2007 (=2009-2) year-class.

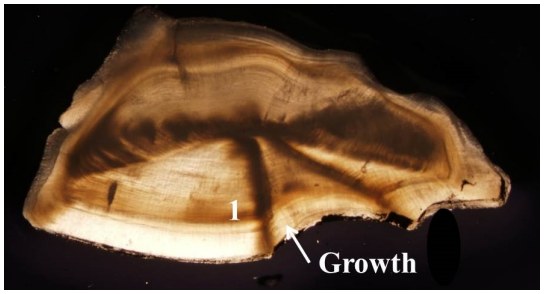


Figure 6: Otolith thin-section from a spotted seatrout showing 1 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is February 2009, before the annulus deposition period. The fish is assigned an age of "1+2" and placed in the 2007 [=2009-2] year-class.

A spotted seatrout taken in May 2009 (during the annulus deposition period) with 4 visible annuli and no translucent growth beyond the last annulus on its thin-section (Figure 7) would be called "4 even" and placed into the 2005 (=2009-4) year-class. The last annulus should fall on the edge, or very close to the edge of the section and have little or no visible extra growth.

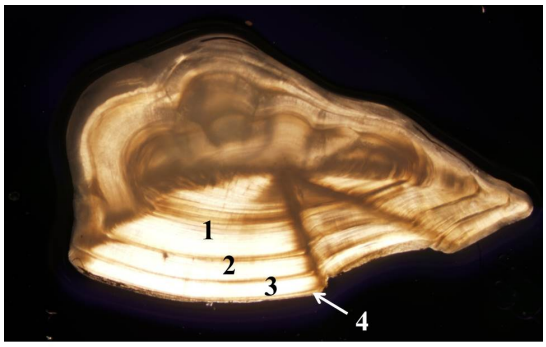


Figure 7: Otolith thin-section from a spotted seatrout showing 4 visible annuli with no translucent growth beyond the last annulus. Its sacrifice date is May 2009, during annulus deposition period. The fish is assigned an age of "4 even" and placed in the 2005 (=2009-4) year-class.

A spotted seatrout taken in August 2009 (after the annulus deposition period) with 4 visible annuli and a small amount of translucent growth beyond the last annulus (Figure 8) would be called "4+4" and put into the 2005 (=2009-4) year-class.

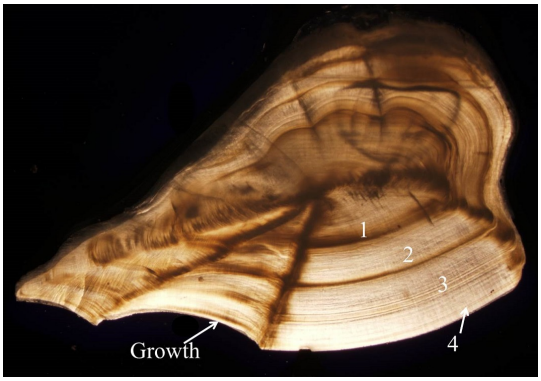


Figure 8: Otolith thin-section from a spotted seatrout showing 4 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is August 2009, after annulus deposition period. The fish is assigned an age of "4+4" and placed in the 2005 (=2009-4) year-class.

Literature Cited

Ihde, T., Chittenden, M. 2003. Validation of presumed annual marks on sectioned otoliths of spotted seatrout, Cynoscion nebulosus, in the Chesapeake Bay region. Bulletin of Marine Science, 72(1):77-87, 2003.

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