

Center for Quantitative Fisheries Ecology

PROTOCOL

AGE ESTIMATION OF OTOLITH TRANSVERSE CROSS-SECTIONS FOR

BLUEFISH

Pomatomus saltatrix

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Introduction

This protocol is for age estimation of bluefish (*Pomatomus saltatrix*) using sagittal otolith transverse cross-sections. For detailed information on how to make a bluefish otolith thin-section slide, please see the protocol for Preparation of Otolith Transverse Thin-sections for Age Estimation of bluefish *Pomatomus saltatrix*. Hereafter, sagittal otoliths will be referred 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 on 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 and 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 bluefish ageing procedure

Ageing bluefish 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 bluefish thin-section from the slide box labeled "Bluefish, VMRC (Year)" (Figure 2).

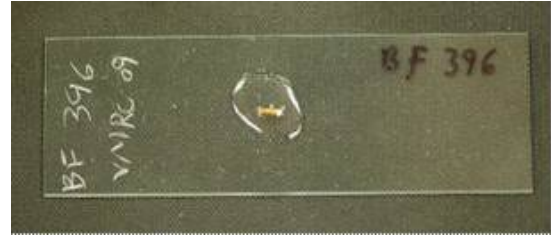


Figure 1: bluefish thin-section mounted on labeled micro slide.



Figure 2: Otolith slide storage box labeled for bluefish.

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 thin-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 thin-section is in clear view (Figure 4). If the thin-section is too large to view, zoom in and examine the core and the sulcal groove (Figure 5). 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 thin-section



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

lacks a clear "V" shape within the sulcal groove, the cut did not go through the core. The otolith must be re-sectioned following the otolith preparation protocol.

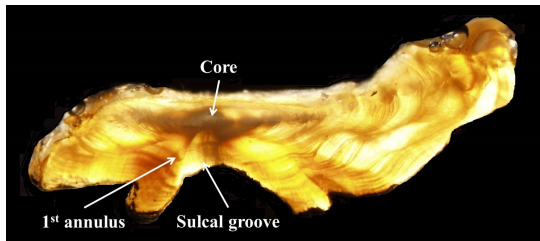


Figure 4: Bluefish thin-section under transmitted light and dark-field polarization.

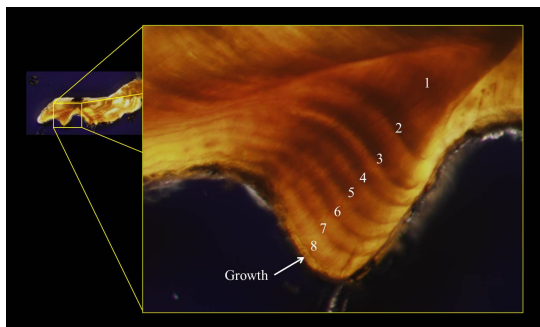


Figure 5: Bluefish thin-section zoomed in for annulus identification.

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.

5. On occasion, the first annulus is difficult to identify because it appears wider and lighter than subsequent annuli (Figure 6). This could be due to temporal differences in the year's earliest and latest hatched fish. After the first annulus, annuli are clear, identifiable, and regularly spaced.

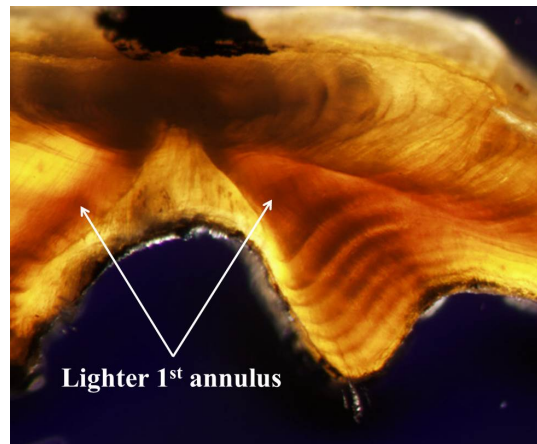


Figure 6: Bluefish otolith thin-section showing a wider and lighter first annulus.

6. Fish that are older than 4 years will occasionally display "double" (Figure 7). A "Double" is characterized by extremely close opaque zones, separated only by a thin translucent zone. It is not known if the "Double" represents 1 or two years of growth because this happens after maturation during periods of less growth. A "Double" is considered to be a single annulus if the opaque zones join at either the edge of the sulcal groove or the dorsal edge of the thin-section (Robillard et al. 2008).

Step 2: Assigning an age to the fish:

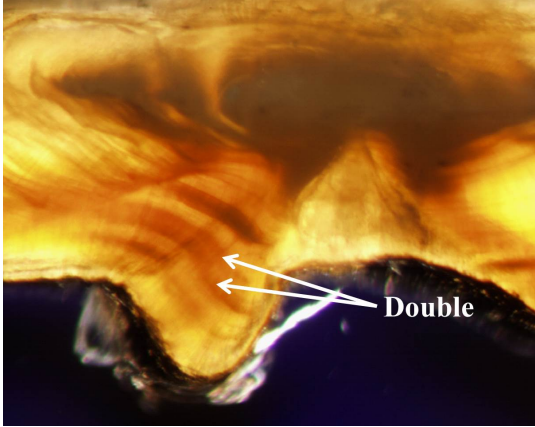


Figure 7: Bluefish otolith thin-section showing a "double".

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 June 30.
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 bluefish annuli are laid down, June 30, 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 June 30 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

Bluefish																								
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
Number of Rings	-	-	-	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	
Assigned Age	-	-	-	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	

Figure 8: bluefish age assigning table

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 8).

A bluefish taken in February 2010 (before the annulus deposition period) with 4 visible annuli and translucent growth beyond the last annulus (Figure 9) would be called "4+5" and put into the 2005 (=2010-5) year-class.

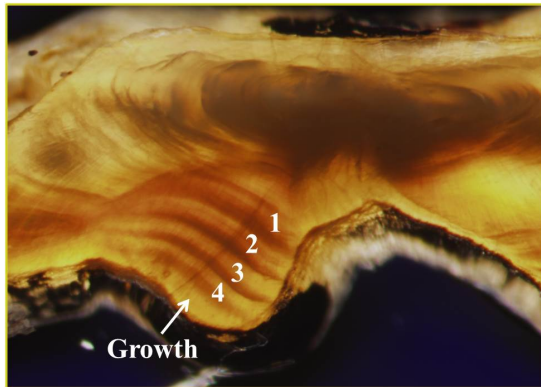


Figure 9: Otolith thin-section from a bluefish showing 4 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is February 2010 (before the annulus deposition period); therefore, we assign "4+5" as its age and place it in the 2005 (=2010-5) year class.

A bluefish taken in May 2010 (during the annulus deposition period) with 5 visible annuli and no translucent growth beyond the last annulus on its thin-section (Figure 10) would be called "5 even" and placed into the 2005 (=2010-5) 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.

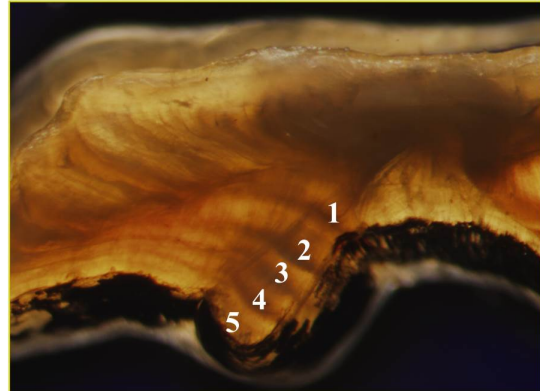


Figure 10: Otolith thin-section from a bluefish showing 5 visible annuli with no translucent growth beyond the last annulus. Its sacrifice date is May 2010 (during the annulus deposition period); therefore, we assign "5 even" as its age and place it in the 2005 (=2010-5) year class.

A bluefish taken in August 2010 (after the annulus deposition period) with 8 visible annuli and a small amount of translucent growth beyond the last annulus (Figure 11) would be called "8+8" and put into the 2002 (=2010-8) year-class.

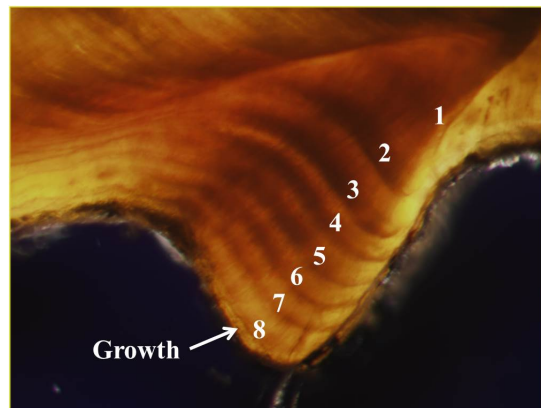


Figure 11: Otolith thin-section from a bluefish showing 8 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is August 2010 (after the annulus deposition period); therefore, we assign "8 + 8" as its age and place it in the 2002 (=2010-8) year class.

Literature Cited

Robillard, E., Reiss, C.S., Jones, C.M.
2008. Age-validation and growth
of bluefish (*Pomatomus saltatrix*)
along the East Coast of the United
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