

Center for Quantitative Fisheries Ecology

## PROTOCOL

# AGE ESTIMATION OF OTOLITH TRANSVERSE CROSS-SECTIONS FOR

## BLACK DRUM

*Pogonias cromis*



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## Introduction

This protocol is for age estimation of black drum (*Pogonias cromis*) using sagittal otolith transverse cross-sections. For detailed information on how to make a black drum otolith thin-section slide, please see the protocol for Preparation of Otolith Transverse Thin-sections for Age Estimation of black drum *Pogonias cromis*. 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 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 black drum ageing procedure

Ageing black drum 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 black drum thin-section from the slide box labeled "Black drum, VMRC (Year)" (Figure 2).



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



Figure 2: Otolith slide storage box labeled for black drum.

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.

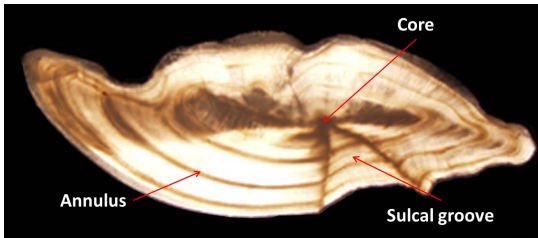


Figure 4: Black drum thin-section under transmitted light and dark-field polarization.

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: May 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 black drum 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 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 black drum taken in March 2009 (before the annulus deposition period) with 10 visible annuli and translucent growth beyond the last annulus (Figure 6) would be called "10+11" and put into the 1998 (=2009-11) year-class.

A black drum taken in June 2009 (during the annulus deposition period) with 10 visible annuli and no translucent growth be-

yond the last annulus on its thin-section (Figure 7), would be called "10 even" and placed into the 1999 (=2009-10) 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.

A black drum taken in August 2009 (after the annulus deposition period) with 11 visible annuli and a small amount of translucent growth beyond the last annulus (Figure 8), would be called "11+11" and put into the 1998 (=2009-11) year-class.

## Photographs

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Black Drum																											
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	2	2	2	2	2	2
Assigned Age	-	-	-	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2

Figure 5: black drum age assigning table

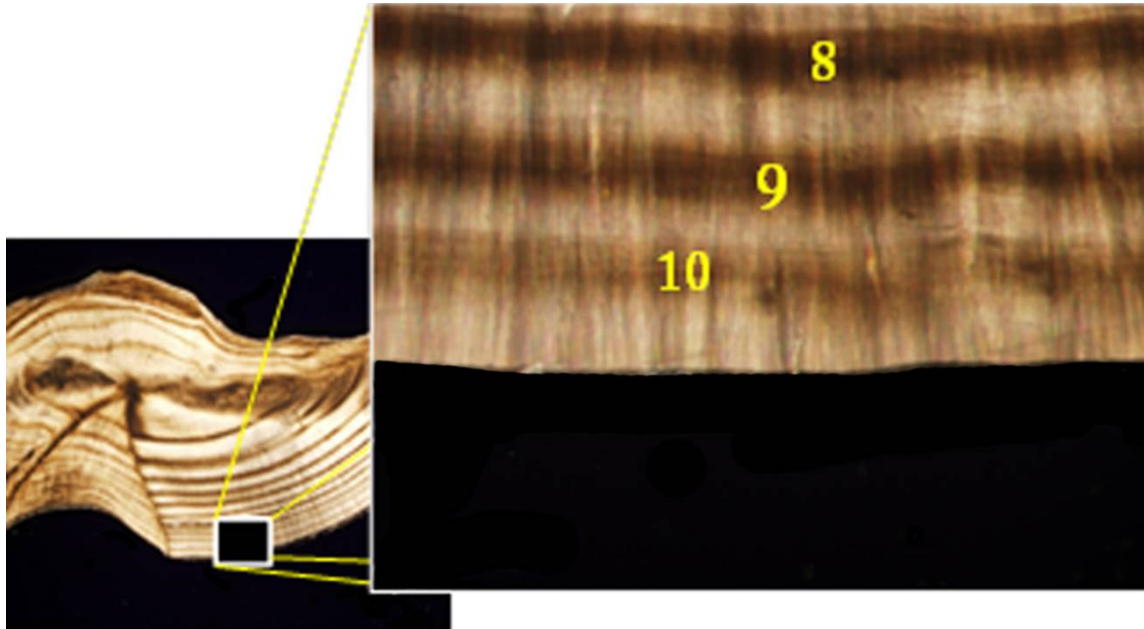


Figure 6: Otolith thin-section from a black drum showing 10 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is March 2009, before the annulus deposition period. The fish is assigned an age of "10+11" and placed in the 1998 (=2009-11) year class.

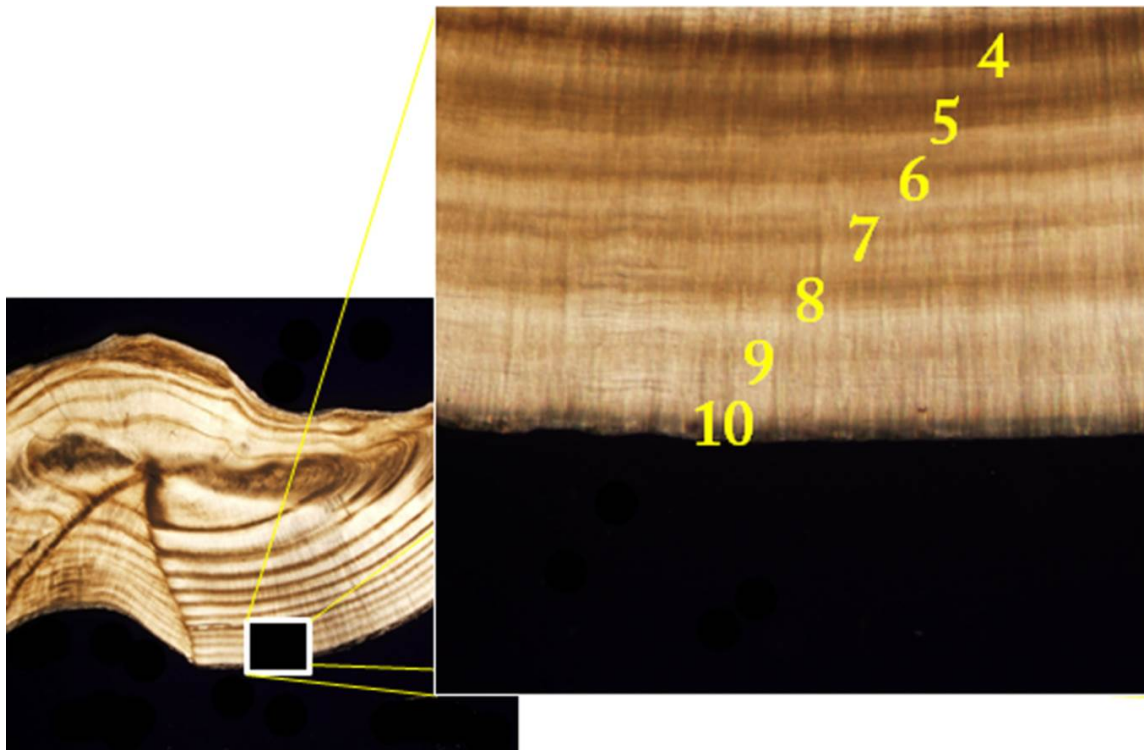


Figure 7: Otolith thin-section from a black drum showing 10 visible annuli with no translucent growth beyond the last annulus. Its sacrifice date is June 2009, during the annulus deposition period. The fish is assigned an age of "10 even" and placed in the 1999 (=2009-10) year class.



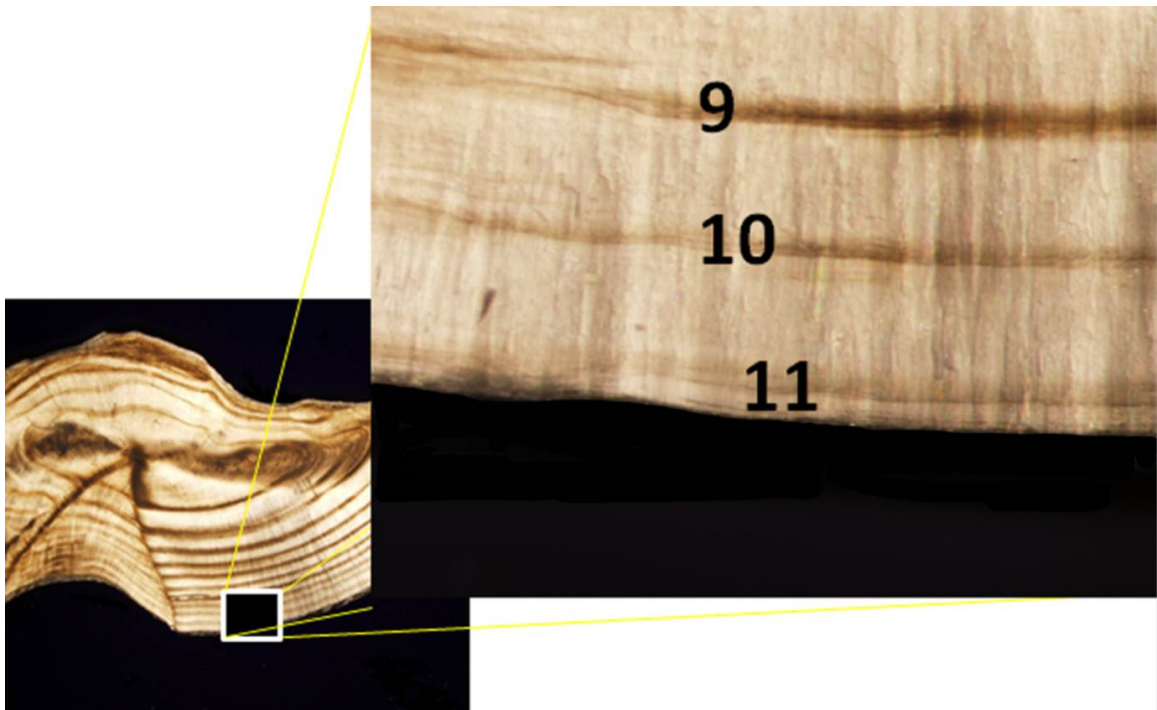


Figure 8: Otolith thin-section from a black drum showing 11 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is August 2009, after the annulus deposition period. The fish is assigned an age of "11+11" and placed in the 1998 (=2009-11) year class.