VMRC Cobia otolith ageing protocol

VMRC

SEDAR28-RD25

8 February 2012



This information is distributed solely for the purpose of pre-dissemination peer review. It does not represent and should not be construed to represent any agency determination or policy.

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Introduction

This protocol is for age estimation of cobia (*Rachycentron canadum*) using sagittal otolith transverse cross-sections. This protocol is to be completed after completion of the protocol for Preparation of Otolith Transverse Cross-sections for Age Estimation of Cobia (*Rachycentron canadum*). Hereafter, sagittal otolith will be referred to as "otolith" and otolith transverse cross-section will be referred to as "thin-section".

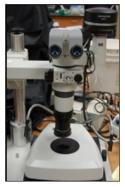




FIGURE 1A

FIGURE 1B

FIGURES 1A & 1B
Nikon SMZ 1000 stereo microscope with 1x objective
and dark-field polarization

GENERAL COBIA AGEING PROCEDURE

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

Specific cobia ageing procedure

Ageing cobia 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

1.1 Remove a labeled slide (*Figure 2*) with mounted tautog thin-section from the slide box labeled "Cobia, VMRC (Year)" (*Figure 3*).



FIGURE 2
Cobia thin-section mounted on labeled micro slide.



FIGURE 3
Otolith slide storage box, labeled for cobia.

1.2 Place the slide on the microscope stage and turn on the transmitted light source. Adjust the dark-field polarization (*Figure 1B*) 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.
- 1.3 Adjust the coarse and fine focus until the entire 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 and the otolith should be re-sectioned following the Protocol for Preparation of Otolith Transverse Cross-sections for Age Estimation of Cobia (*Rachycentron canadum*).
- 1.4 When a good section has been obtained, count each visible annulus on the section starting from the core, moving towards the proximal edge. An otolith annulus is the combination of an opaque and translucent band, however, when ageing otoliths, only the opaque lines are counted. 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:

- 2.1 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: June 1 to June 30 (Richards, C.E. 1967).
- 2.2 There is growth beyond the last annulus: The growth is indicated by a "+" after the number of annuli, that is "X+".
- 2.2.1 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 cobia annuli are laid down (July

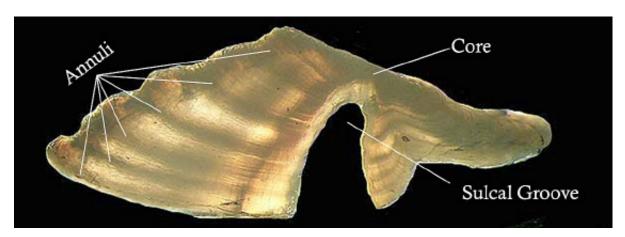


FIGURE 4
Cobia thin-section under transmitted light and dark-field polarization.

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- 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 July 31 and before January 1, the fish has laid down its annulus for the year and has experienced growth since that time. The age of the fish is represented as "X + (X)". A fish with 6 annuli visible on its otolith trans-verse cross-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 ageing data found in Figure 4.

A cobia taken in May 2011 with 5 visible annuli and translucent growth beyond the last annulus (*Figure 5*) would be called "5 + 6" and would be put in the 2004 (current year – 6) year-class.

If a cobia is taken in June 2011 (during the annulus deposition period) and its thin-section has 4 visible annuli (*Figure 6*), it would be called "4 even" and would be placed into the 2007 (current year – 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.

If a cobia is taken in July 2011 (after the annulus deposition period) and has 4 visible annuli with a small amount of growth beyond the last annulus (*Figure 7*), it is said to be 4 + 4 and it is put in the 2007 (current year -4) year-class.

Cobia Thin-section Ageing

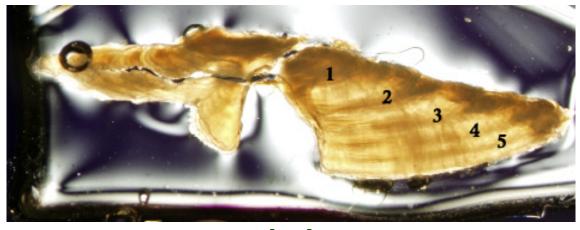


FIGURE 5
Otolith thin-section from a cobia showing 5 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is May 2011 (before the annulus deposition period); therefore, we assign "5 + 6" as its age and place it in the 2005 [current year-6] year class.

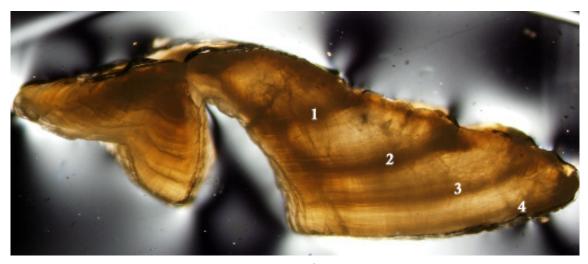
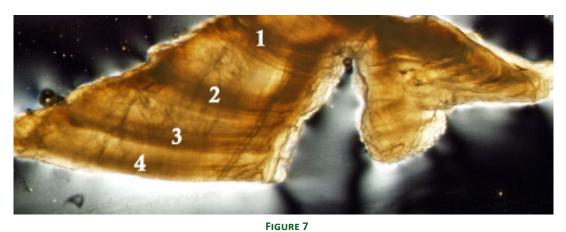


FIGURE 6
Otolith thin-section from a cobia showing 4 visible annuli with translucent growth beyond the last annulus. Its sacrifice date is June 2011 (during the annulus deposition period); therefore, we assign "4 even" as its age and place it in the 2007 [current year-4] year class.



Otolith thin-section from a cobia showing visible annuli with translucent growth beyond the last annulus. Its sacrifice date is July 2011 (after the annulus deposition period); therefore, we assign "4 +4" as its age and place it in the 2007 [current year-4] year class.

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0 8 8 0 Z N N N Year Three 1/1 Year Two 1/1 Year One 1/1 Cobia Rachycentron canadum ssigned birth date

LITERATURE CITED

Richards, C.E. 1967. Age, Growth, and Fecundity of the Cobia, *Rachycentron canadum*, from Chesapeake Bay and Adjacent Waters. Contribution No. 252, Virginia Institute of Marine Science, Gloucester Point, Virginia, 343-350.

PHOTOGRAPHS

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PROTOCOL

Using otolith TRANSVERSE CROSS-SECTIONS FOR AGE ESTIMATION OF

Cobia Rachycentron canadum