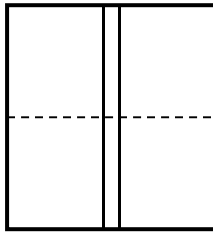


Winter Flounder Otolith Cutting and Reading Protocol



1. Determine which otolith is the top otolith by looking at both otoliths under a scope with transmitted light. The nucleus of the top otolith will be in the center rather than offset slightly as is the bottom otolith.
2. Mount otolith on an otolith cutting sheet with the succal groove down and the nucleus facing you. Completely cover otolith with Crystal Bond to assure the transverse section stays complete after sectioning.
3. Cut using 3" Buehler Diamond Wafering Blades. Take a thick section through the center of the otolith. Cut the section so that the nucleus is at one side of the section (ie. Do not put nucleus in center of section). Mount this section (nucleus side down) on a microscope slide using a thin layer of crystal bond. Wet sand the section using 400 to 1200 grit sand paper to an appropriate thickness so that the annuli can be easily read. Once the section is sanded, it can then be covered with a layer of crystal bond.



Example of an otolith cutting sheet, with correct orientation. Place otolith on sheet so that the nucleus is on one of the two center lines.



The above otolith is Age 3.

4. Otoliths are read by counting each annulus, or dark ring. Spawning and annuli deposition occur in the late winter and early spring for the Mid-Atlantic and Southern New England Regions. Samples collected from the spring NEAMAP survey would have a recently formed annulus, Mark Formed Recently, MFR, an annulus forming on the edge (Dark), or an annulus about to form, Mark Not Visible, MNV. The MNV comment depicted as a dark edge will occur when the last visible annulus has some light/translucent growth after it before reaching the edge of the otolith.
5. All otoliths should be read by 3 independent readers. The mode of the 3 reader is evaluated by a senior reader to establish the final age assignment.

