

Improving the precision of otolith-based age estimates for Greenland halibut (*Reinhardtius hippoglossoides*) with preparation methods adapted for fragile sagittae

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Otolith-based age estimates for Greenland halibut (*Reinhardtius hippoglossoides*) have low precision, and there is general uncertainty about their accuracy in older fish (Anon.¹; Alpoim et al.²). Low precision can result from inadequate training of age readers, poor aging criteria, or peculiarities of the structure being aged (Kimura and Lyons, 1991). The latter is the primary cause of low precision with Greenland halibut, and it confounds attempts to improve the former two. Sagittae of Greenland halibut are irregular in shape and exhibit marked bilateral asymmetry

(Fig. 1). Much of this irregularity is due to finger-like projections, which begin as small, marginal tubercles in 4- to 6-year-old fish, and can develop into convoluted, fragile structures in older fish. The variable deposition rate of aragonite and protein that produces these structures makes interpretation of growth patterns difficult and results in age estimates that vary depending on which region of the otolith is examined.

The amphiboreal distribution of Greenland halibut has led to their exploitation by the industrial fisheries of more than ten nations in the North Atlantic and North Pacific Oceans and by several aboriginal fisheries in the near shore regions of Greenland and northern Canada (Alton et al., 1988; Witherell³; Anon.⁴; Treble⁵). Age determination (aging) and age structure analysis have been undertaken primarily for North

Atlantic and Barents Sea stocks. Methods vary between laboratories but the majority of aging is accomplished by examining the surface patterns of whole sagittae. For the purposes of this note, "surface" (or "surface aging") will refer to the surface pattern of the whole sagitta. Generally, only the left (i.e., blind side of fish) sagitta is aged because it has a more centric nucleus, resulting in more evenly spaced annuli (Lear and Pitt, 1975; Bowering, 1978, 1982; Haug and Gulliksen, 1982; Anon.¹; Bowering and Nedreaas, 2001; Alpoim et al.²). Attempts to improve the resolution of growth patterns have included baking both sagittae, clearing them with oil, grinding the distal surface of the left sagitta, and breaking and burning the left sagitta (Anon.¹; Kuznetsova et al., 2001; Alpoim et al.²). To date these processes have had equivocal effects on the precision of age estimates. International exchanges of Greenland halibut otoliths have yielded mixed results; reported between-reader agreement (± 0 year) has ranged from 1% to 69% (Anon.¹) and from 37% to 51% (CVs ranging from 5.81% to 9.58%) (Alpoim et al.²). Despite these exchanges, concern about precision still exists and a consensus on preferred aging methods for Greenland halibut has not been reached.

The Alaska Fisheries Science Center (AFSC) has collected Greenland halibut otoliths from the Bering Sea and Aleutian Islands for over 20 years, but little aging was attempted prior to 2003. Initial examination of the otoliths left AFSC age readers

¹ Anonymous. 1997. Report of the ICES/NAFO workshop on Greenland halibut age determination, Reykjavik, Iceland, 26–29 November 1996. ICES CM 1997/G:1, 53 p. Palaegrade 2-4 DK-1261 Copenhagen K Denmark.

² Alpoim, R., E. Roman, B. Greene, R. Burry, and W. R. Bowering. 2002. Results of the Greenland halibut (*Reinhardtius hippoglossoides*) otolith exchange between Spain, Canada, and Portugal. In NAFO Scientific Council Meeting, June 2002. NAFO SCR Doc. 02/141, 14 p. P.O. Box 638, Dartmouth, Nova Scotia, Canada B2Y 3Y9.

³ Witherell, D. 2000. Groundfish of the Bering Sea and Aleutian Islands area: species profiles 2001. North Pacific Fisheries Managements Council, 605 W. 4th Ave. Ste. 306 Anchorage, AK 99501-2252.

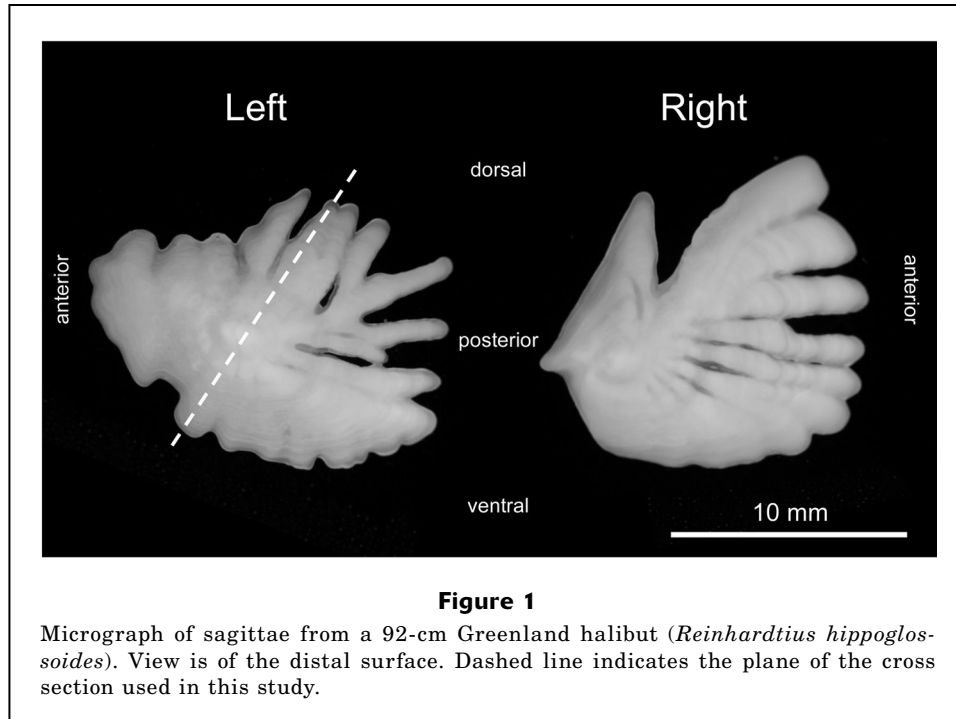
⁴ Anonymous. 2004. Annual quota table for 2005. In 2004 Annual report of the Northwest Atlantic Fisheries Organization, P.O. Box 638, Dartmouth, Nova Scotia Canada B2Y 3Y9.

⁵ Treble, M. 2005. Personal commun. Arctic stock assessment biologist, Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, Manitoba Canada R3T 2N6

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with a general lack of confidence in surface age estimates. This uncertainty, coupled with the observation that otoliths of larger, presumably older, fish tend to grow in thickness rather than in sagittal diameter, led to pilot work for processing and production (large-scale) aging of Greenland halibut sagittae. Various methods reported in the literature and several new techniques were explored. This pilot work converged on a method that involved cutting the left sagitta in the transverse plane and staining the two resulting cross sections. This method is similar to the break-and-burn method (Chilton and Beamish, 1982) but is more amenable to fragile Greenland halibut sagittae. The goal of the present study was to determine whether the precision of Greenland halibut age estimates could be improved by examining the stained cross sections of their sagittae rather than the surfaces of the whole sagittae, and to determine whether there was a significant difference in age estimates made with each of the two methods.

Materials and methods

Otoliths were collected from Greenland halibut in July 1998 and June–August 1994 as part of the AFSC Bering Sea and Aleutian Islands trawl surveys. Sagittae were removed at sea and stored in a glycerol-thymol solution until the time of our study. Fish length (TL) was measured to the nearest centimeter for each specimen.

Surface aging was accomplished by submerging sagittae in water over a black background and counting probable annuli (i.e., translucent zones) with a dissecting microscope (6–25 \times) with reflected light. Probable

annuli were counted along several vectors on both sides of the right and left sagittae. A count that was repeatable, with highest reader confidence, was adopted as the surface age estimate.

The left sagitta (i.e., on blind side of fish) was embedded in clear polyester resin and cut into two pieces with a low-speed saw. The cut was made slightly obliquely to the transverse plane and was adjusted for each otolith to ensure that the saw blade bisected the nucleus, passed through a thick section of the perisulcular region (i.e., a portion with a large mediolateral dimension), and extended out the center of a prominent dorsal finger (Fig. 1). The two exposed cross sections were then polished with 800-grit wet-dry sandpaper on a lapidary wheel to remove saw marks.

Staining techniques were adapted from Richter and McDermott (1990). Polyester blocks containing cut otoliths were submerged in a solution of 1% Aniline Blue WS (no. B362-03, Mallinckrodt Baker Inc., Phillipsburg, NJ) in 1% acetic acid. Staining times varied from 10 to 15 minutes initially and were consolidated to 13 minutes as the experiment progressed. Stain solution temperature was maintained between 20° and 23°C. Upon removal from the stain, otoliths were rinsed with fresh water and wiped clean to ensure that residual acid and stain were removed. The two cross sections were covered with mineral oil to eliminate glare, and were examined under a dissecting microscope at 12 \times to 50 \times magnification with reflected light. Blue stained translucent zones (Fig. 2) were counted, and this number was adopted as the cross-section age estimate.

Three trials were conducted to examine the possible benefits of cutting and staining Greenland halibut sagit-

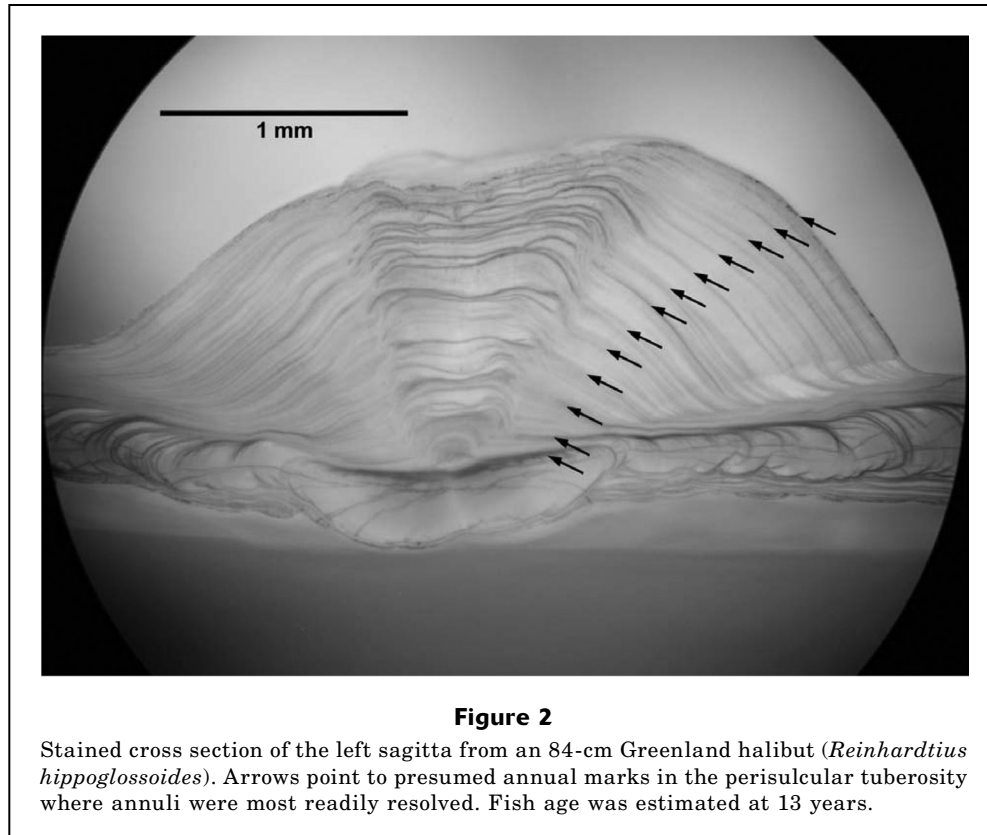


Figure 2
Stained cross section of the left sagitta from an 84-cm Greenland halibut (*Reinhardtius hippoglossoides*). Arrows point to presumed annual marks in the perisulcular tuberosity where annuli were most readily resolved. Fish age was estimated at 13 years.

tae. In trial 1, a training trial, sagittae of 93 Greenland halibut were examined to compare precision between the two aging methods and to calibrate age readers with respect to the first few annuli on the stained cross sections. This sample included fish that ranged from 12 to 84 cm TL, but it was dominated by smaller fish (mean TL=40 cm, median=31 cm). Two readers independently aged sagittal surfaces and stained cross sections. Surface aging necessarily preceded cutting and staining, but no consultation occurred between readers until the end of the trial. Readers were aware of fish length during aging. At the end of trial 1 the independently determined age estimates were compared and readers re-examined otoliths that had resulted in age discrepancies.

In trial 2, 226 otolith pairs were examined. This sample contained sagittae from many larger specimens (mean TL=75 cm, median=72 cm, and range=57 to 98 cm). Ages were determined in the same manner as in trial 1. However, after surface aging, readers re-examined discrepancies together and assigned, by mutual agreement, a definitive surface age to each sagitta prior to cutting and staining. Similarly, the cross sections were aged independently and then assigned a definitive cross-section age by mutual agreement. This process is similar to that used for production aging of other species at the AFSC (Kimura and Lyons, 1991) and allowed not only a comparison of precision between methods but also a comparison of the final age estimates that resulted from the two methods. In trial 3, sagittae were exam-

ined from 76 Greenland halibut with a size range of 12 to 63 cm TL (mean=37 cm, median=39 cm). This trial was conducted in the same manner as trial 2, with the exception that fish length was not provided to the readers. We felt that criticism could arise if length data were known because of the potential for reader bias when aging small fish that fall into distinct size classes.

Two age readers performed each trial. Reader 1 was relatively inexperienced with six months of experience aging larval otoliths and one month of experience aging adult otoliths. Reader 2 had 14 years of experience aging several species, including other Bering Sea flatfishes. Neither reader had previously aged Greenland halibut.

Between-reader agreement and coefficient of variation (CV) were calculated for each aging method from each trial. CV was used as the measure of precision (Chang, 1982). Percent agreement is not a good measure of precision because it is highly dependent on the age structure of the sample. Bowker's test of symmetry (Hoenig et al., 1995) was used to assess between-reader bias. Definitive ages from trials 2 and 3 were compared by using a two-tailed matched pairs *t*-test (Snedecor and Cochran, 1967). Von Bertalanffy growth parameters were estimated from surface and cross-section ages combined from trials 2 and 3. An *F*-test based on the residuals of nonlinear least-squares fit was used to test for difference between the resulting models (Quinn and Deriso, 1999).

Results

Stained cross sections improved the precision of Greenland halibut age estimates for the larger, presumably older, specimens in trial 2 but did not improve precision of estimates for specimens in trials 1 and 3. Percent CVs were 11.33, 16.31, and 8.11 for surface ages and 19.68, 9.64, and 9.96 for cross-section ages from trials 1, 2, and 3, respectively (Table 1). A similar pattern occurred in the symmetry of age estimates. Bowker's test of symmetry indicated that surface age estimates in trial 2 were significantly biased between age readers ($P < 0.0342$) and that cross-section estimates were not ($P < 0.2159$), whereas in trials 1 and 3 significant bias occurred in the cross-section estimates ($P < 0.0001$ and $P < 0.0012$, respectively) (Table 1).

These equivocal results were primarily caused by difficulty interpreting the second annuli on cross sections. Reader 1 tended to count a small diameter mark close to the nucleus as the second year whereas reader 2 considered it a check. A *post hoc* correction of this bias in trial 1 (i.e., adding 1 year to each of reader-2's cross-section estimates) yielded better precision (CV=7.68) and no significant bias ($P < 0.2440$) (Table 1). This problem in interpretation occurred in all trials but the resulting bias was most noticeable in trials 1 and 3 where fish age estimates were younger.

Definitive cross-section ages were significantly greater (older) than definitive surface ages for trial 2

($t = 17.32$, $df = 225$, $P < 0.0001$). Mean cross-section age was 17.1 years and had a range from 9 to 36 years, whereas mean surface age was 12.4 years and had a range from 7 to 28 years (Fig. 3A). Differences between definitive cross-section ages and definitive surface ages in trial 3 were not significant ($t = 1.74$, $df = 74$, $P < 0.0858$). Mean stained age was 4.29 years and had a range from 1 to 7 years, and mean surface age was 4.15 years and had a range from 1 to 8 years (Fig. 3B). Von Bertalanffy growth parameters calculated from the definitive surface ages (trial 2 and 3 combined) were $L_{\infty} = 103.7$, $K = 0.104$, and $t_0 = -0.333$. Parameters from definitive cross-section ages were $L_{\infty} = 86.2$, $K = 0.125$, and $t_0 = -0.233$. The models varied significantly from each other ($F = 40.58$, $P < 0.0001$) (Fig. 4).

Discussion

In larger Greenland halibut (i.e., in trial 2), the precision of age estimates can be improved by aging stained cross sections of sagittae rather than aging sagittal surfaces (Table 1). The sagittae of larger, older Greenland halibut are very difficult to interpret from the surface. Marginal growth increments are very small and are interrupted by the fingerlike projections on the otoliths. Age readers in our study were more confident in the age estimates they made from stained cross sections. The clearest annuli were encountered in the perisulcular region of left sagittae. This region appears to grow more consistently than other areas of the otolith. Staining allowed resolution of very narrow increments in this region that were not visible on the surface of sagittae.

Precision did not increase in trials 1 and 3 (Table 1) because these trials contained many smaller specimens. The benefits of cross-sectioning and staining are not as great in otoliths that are still growing rapidly in sagittal diameter. The magnitude of the difference in age estimates from whole surfaces and stained cross sections did not exceed 1 year in fish less than 46 cm and did not exceed 2 years in fish less than 57 cm. A second confounding factor was interpretation of the second annuli in cross sections. This consistent one-year bias between readers outweighed any improvements that may have resulted from cross-sectioning otoliths in smaller specimens. We feel that more interreader calibration and validation of cross-sectioned annuli by the Peterson method (Ricker, 1975) can resolve this problem.

The increase in precision in trial 2 was accompanied by age estimates that were significantly greater (older) (Fig. 3A). In 24 cases, the cross-section estimate was 10 or more years greater than the surface age estimate, and in two cases the cross-section estimate was 22 years greater. The mean

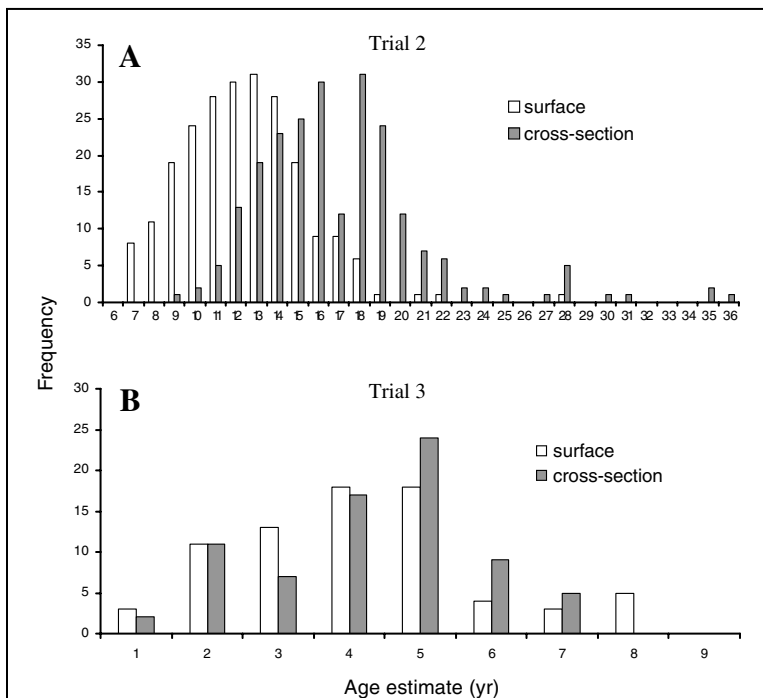


Figure 3

Age frequencies for age estimates from sagittal surfaces and from sagittal cross sections of Greenland halibut (*Reinhardtius hippoglossoides*) in trial 2 (A) and trial 3 (B) of this study.

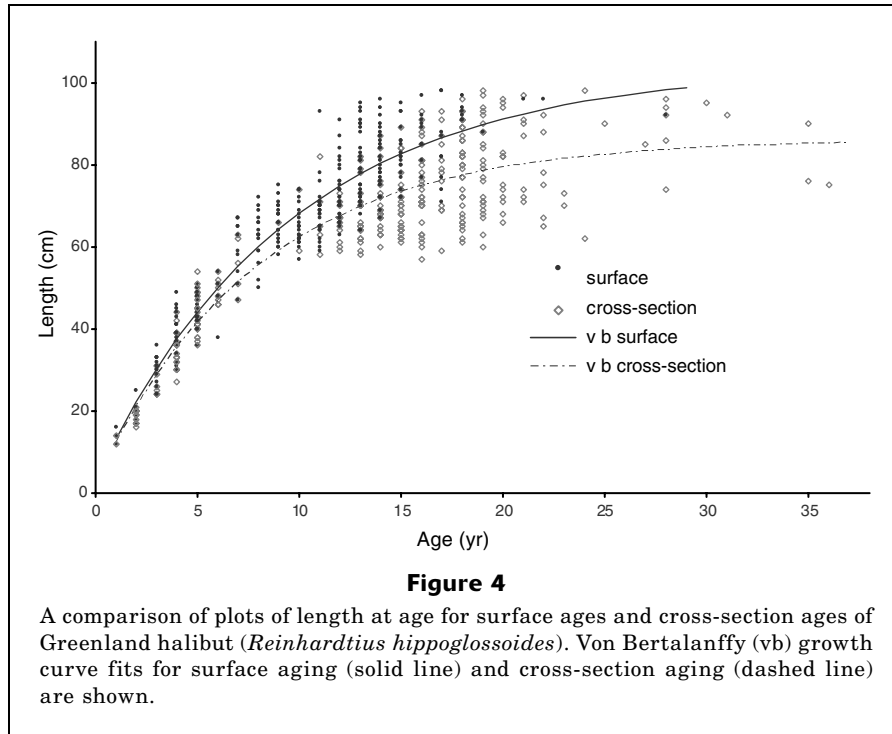


Table 1

Precision of age estimates made from the surfaces and cross-sections of Greenland halibut (*Reinhardtius hippoglossoides*) sagittae. Data from three trials with mean fish length and collection location indicated. BS = Bering Sea, AI = Aleutian Islands.

	n	Collection year, location	Mean fish length (cm)	Ageing method	Percent CV ¹	Between reader % agreement			Bowker's test of symmetry ²		
						±0 yr	±1 yr	±2 yr	χ ²	df	P<
Trial 1	93	1998, BS	40	surface	11.33	44.1	81.7	90.3	30.4	22	0.1091
				cross-section	19.68	20.4	66.7	81.7	74.0	28	0.0001
				cross-section corrected ³	7.68	53.8	81.7	93.5	27.3	23	0.2440
Trial 2	226	1994, AI	75	surface	16.31	16.8	42.0	58.4	94.2	71	0.0342
				cross-section	9.46	16.3	44.3	68.1	76.9	68	0.2159
Trial 3	75	1994, AI	37	surface	8.11	65.3	89.3	93.3	19.5	11	0.0532
				cross-section	9.96	49.3	90.7	98.7	32.3	12	0.0012

¹ Percent CV = coefficient of variation × 100; CV calculated following Chang (1982).

² P<0.05 from Bowker's test indicates asymmetry of discrepancies between readers (i.e., reader bias).

³ Corrected cross-section statistics from trial 1 are the result of a *post hoc* adjustment of reader 2's ages (see text).

age estimate increased by 4.7 years for 226 fish with mean length of 75 cm. The oldest surface age estimate was 28 years and the oldest cross-section estimate was 36 years (Fig. 3A). Maximum ages of Greenland halibut reported in the literature rarely exceed 20 years (Alton et al., 1988). These older ages result in smaller size-at-age and have the effect of decreasing estimates of von Bertalanffy's L_{∞} (Fig. 4).

The older cross-section age estimates for Greenland halibut are consistent with natural mortality estimated by the gonadosomatic index method ($M=0.112$; Cooper et al., in press). Our maximum cross-sectioned age of 36 years indicates $M = 0.115$, as opposed to an $M = 0.149$ as indicated by the maximum surface age of 28 years (Hoenig, 1983). Current natural mortality parameters used in management are 0.18 in the Bering Sea and

Aleutian Islands (Ianelli et al.⁶) and 0.20 in the North Atlantic (Darby et al.⁷). These values are more consistent with surface age estimates.

We feel that age estimates made from stained cross sections are an improvement over surface age estimates for Greenland halibut. However, validation of the age estimates produced by these methods is necessary. Given the large discrepancies that we encountered in some specimens (10 to 20 years) these ages can be roughly tested with tag-recovery or radiometric methods.

The methods used in the present study may have application in other hard-to-age species. They are a practical alternative to serial thin sections because the preparation time is shorter and allows the method to be adapted for production aging. The embedding process also preserves the structure of fragile otoliths which can be damaged during the cutting and break-and-burn processes.

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