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MANAGEMENT BRIEF

Comparisons of Precision and Bias with Two Age Interpretation Techniques for Opercular Bones of Longnose Sucker, a Long-Lived Northern Fish

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Abstract
Two preparations of opercular bones for estimating age of longnose suckers *Catastomus catastomus* revealed that annuli were obscured by dense bone and undetected when the whole operculum technique was used. By thin-sectioning the opercula, we removed the dense bone, revealing previously obscured annuli. Using opercula collected from older fish in Labrador, Canada, we found that the dense bone overgrowth led to an overall age bias of 1 year. When samples were broken into groups based on sectioned ages, however, there were minimal differences in age between the two techniques for fish age 6 and younger and a 2-year age difference for fish estimated to be age 10 and older. To compare precision in locating annuli between the two techniques, we calculated coefficient of variation values among independent determinations. Both techniques demonstrated low variance, however, age determinations had greater variation with thin-sectioning than with whole opercula interpretations. Therefore, we conclude that care must be taken when making annulus determinations from thin-sectioning. Due to the presence of dense bone overgrowth, associated with the whole operculum technique, we conclude a combination of both techniques would provide the most thorough procedure for interpreting opercular age for such long-lived fish (30–50 years).

Longnose suckers *Catastomus catastomus* are common throughout all provinces and territories in Canada. Both young and adults are important prey for several sport fish including northern pike *Esox lucius* and lake trout *Salvelinus namaycush* (Scott and Crossman 1973). This species is long-lived (>40 years) and is plentiful in lakes of southwestern Labrador but becomes less abundant from west to east. Opercula from longnose suckers were collected from two areas of the upper Churchill River drainage basin in Labrador. Lakes in this region of Labrador are of low productivity (Duthie and Ostrofsky 1974), experience short growing seasons, and have little fishing pressure, characteristics that tend to produce stable population structures (Johnson 1976; Power 1978). Therefore, longnose suckers collected from this area were expected to grow slowly and to live long.

When working with whole opercula of longnose suckers, we observed a possible source of error that could create bias and affect precision in age determination. Dense bone found on the surface of opercula from older suckers affected light transmission, making it difficult to discern individual annuli. In some instances dense bone appeared to overgrow the origin of the operculum, potentially obstructing the first few annuli. It has been documented that the presence of dense bone can potentially overgrow and obscure increments and several annuli (Casselman 1987). When estimating ages of the catostomid cui-ui *Chasmistes cujus,* Scoppettone (1988) discovered that the support bone, located ventrad of the hyomandibular socket, obscured annuli in older fish. Therefore, in older fish, there is a greater potential for error in age determination. Error associated with dense bone when interpreting opercula was a problem when interpreting age of northern pike (Frost and Kipling 1959) and possibly yellow perch *Perca flavesens* (Le Cren 1947). An alternative method, in which individual samples are thin-sectioned, may aid interpreters in identifying annuli obscured by dense bone.

The purpose of our study was to compare age determinations from whole and sectioned opercula of longnose suckers. We hypothesized that whole opercula interpretations underestimate age relative to sectioned opercula. We also hypothesized that thin-sectioning the operculum bone allows for greater

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light transmission and exposes the annuli under the dense bone, thereby decreasing error in annulus identification and measurement compared with interpretations made from the surface of the whole operculum.

METHODS

Sample collection.—Juvenile and adult longnose suckers were obtained from Lake Joseph ($N = 41$) and Lake Lobstick ($N = 65$). These lakes, located between 53°N and 55°N and 63°W and 66°W on the Labrador plateau, are 400–575 m above sea level and are part of Smallwood Reservoir.

Longnose suckers were captured with monofilament diamond-mesh gill nets that increased in size from 12.7 to 127 mm by 12.7-mm increments. Net panels were attached in series from smallest to largest mesh sizes. Three net gangs were set perpendicular to shore in both lakes.

Fork length, whole weight, sex, date, and location of capture of longnose suckers were documented, and both opercula were collected from all fish. On the collection date, opercula were dipped in warm water, cleaned of all tissue, and dried. To control for possible asymmetric growth differences between left and right opercula, only the left operculum was selected for interpretation. To minimize interpreter bias from recognition of individual fish samples, opercula were assigned serial numbers, and the period between interpretation trials was approximately 1–2 weeks. For each technique (whole and sectioned opercula), four trial interpretations were conducted per fish at different times for a total of eight interpretations. To reduce variation that would normally be associated with several interpreters, a single individual conducted all age determinations.

Whole operculum interpretation.—Each whole operculum was examined in transmitted light under a dissecting microscope equipped with a drawing tube (7× to 14× magnification). Individual annual increments were measured and counted with computer software that extracted and collated the interpreted fish age and growth (Casselman and Scott 2000). To interpret increments, the deeply translucent zones that were bordered by opaque zones were digitized and recorded as annuli. Bones were viewed with the concave side facing up, on a glass plate engraved with a radial line (Marcogliese 1996). The bone was aligned so that the radial line began at the distal edge of the articulation socket and extended to the maximum edge of the anterior dorsal region and was at right angles to the growth increments (Figure 1A). The first radial line was reflected through a drawing tube and overlaid on a second radial line secured to a digitizing pad. The translucent distal edges of interpreted annuli were then digitized where they intersected at right angles with the projected radial line.

With the projected radial line as a guide, the radius of interpretation for the whole operculum technique was marked at three points with a lead pencil. By marking the radius, we could then subsample segments of the radius at higher magnification. It also allowed us to cut the identical radius used in the sectioning technique.

Sectioned operculum interpretation.—The same opercula that were used for whole operculum interpretation were embedded in Araldite epoxy (Casselman and Gunn 1992). Following hardening, a 600-μm-thick transverse section from the frontal plane of the operculum was cut using a low-speed Isomet saw (Buehler Canada Ltd., Toronto, Ontario). The section cut was aligned so that the radial line began at the distal edge of the articulation socket and extended to the maximum edge of the anterior dorsal region, the identical radial line from which the intact structure was first interpreted (Figure 1B). Following cutting, individual embedded sections were mounted on glass slides with the resin epoxy mixture and polished smooth using 800-grit sandpaper in preparation for interpretation. We used the same magnification (7× to 14×) criteria and process for enumeration and quantification of increments as outlined in the whole operculum technique.

Analysis.—To compare the reproducibility of age determinations between techniques, coefficient of variation (Chang 1982) values were calculated among the four reading trials for each fish and technique as

$$CV_{i,j} = (SD_{i,j}/\text{mean}_{i,j})100,$$

where the mean and standard deviation (SD) are summary values for the four measurements on individual i and method $j = 1$ or 2. We compared the resulting CV values for each technique using a paired t-test, which is generally used when measurements are taken from the same subject before and after some manipulation. In this instance, the operculum bone is the subject, and the treatment is the sectioning of the individual bones. By pairing age determinations in this manner we accounted for differences that were inherent within individual bones, and therefore, any remaining difference could be attributed to the technique applied.

Using fish age 11 and older, we recorded distances from origin to the first five and the last five annuli. These measurements were performed four times for each technique (four times on each whole operculum and four times on each sectioned operculum). We chose fish age 11 and older to avoid duplication when measuring for each annulus group. Using the four measurements, we calculated the mean distance to each growth mark, giving us two mean values per growth mark and per sample. The mean distance to each annulus provided a precision comparison between the two techniques for the pattern of bone growth when the fish were young and when the fish were older.

To test precision in relocating the exact position of individual annuli, we calculated CV values for each of the 10 annuli positions. We then repeated the use of the paired t-test on mean CV values for the first five annuli and the last five annuli between techniques ($\alpha = 0.05$).

The paired t-test was also used to evaluate whether there was an overall age difference between techniques. Individual

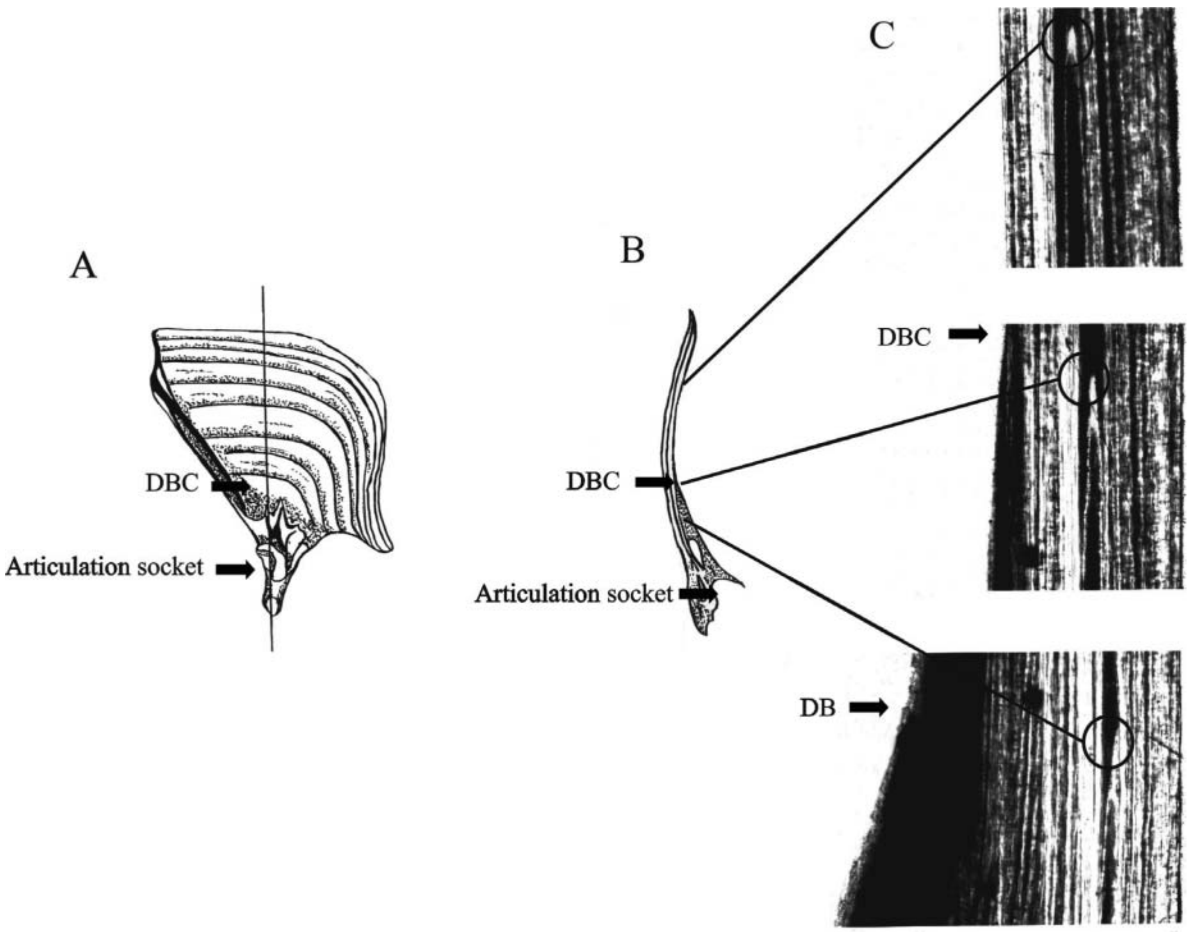

FIGURE 1. Schematic diagram showing placement of radial line on **(A)** a whole longnose sucker operculum and **(B)** the operculum cross-section, with extrapolations to **(C)** photographs showing the annuli at locations corresponding to their position in the operculum, where the distal extent of dense bone (DBC) and dark margin of dense bone (DB) are noted. Open circles indicate position of annuli.

samples were paired by interpretative trial and sample number, where sample 1 from trial 1 of the whole operculum technique was paired with sample 1 from trial 1 of the sectioning technique. Pairing the samples in this manner accounted for variance associated with sample familiarity due to repeated trials and variance in aging that may exist due to individual sample quality.

To determine at what age dense bone overgrowth influenced interpretations, samples were broken into subgroups. Using measurements made during trial 1 of the sectioned method, we created subgroups for all trial age assessments. Subgroups were created based on the relative difference in measured distances between the origin and the distal extent of dense bone, and the distance from the origin to the first increment (Figure 2). Samples were placed in subgroup 1 if the length of dense bone was shorter than the distance to the first increment and in subgroup 2 if the dense bone length was longer than the distance to the first increment. Using this comparison, samples were split into two age subgroups: those that had not been affected by dense bone overgrowth (age 6 and younger) and those that we identified as having at least one annulus obscured by dense bone overgrowth (age 10 and older; Figures 1C, 2B). Paired t-tests were used to determine whether there were differences between subgroups ($\alpha = 0.05$).

To further compare age bias, we averaged the ages assigned across the four trials and created an age-bias plot comparing the sectioned technique to the whole operculum technique (Campana et al. 1995). All statistical tests were done using the SPSS statistical package, version 11.5.1.

RESULTS

In total, 106 fish were examined. During processing, 11 samples were damaged, reducing our sample size to 95.

Both techniques demonstrated low variance; however, age determinations had greater variation with thin-sectioning (CV = 8.0%) than with whole-operculum interpretations (CV = 3.5%). In accordance with the low mean coefficient of variation values, both techniques gave good results for the repeatability of age determination. However, the whole-operculum technique gave significantly greater interpretive repeatability than the

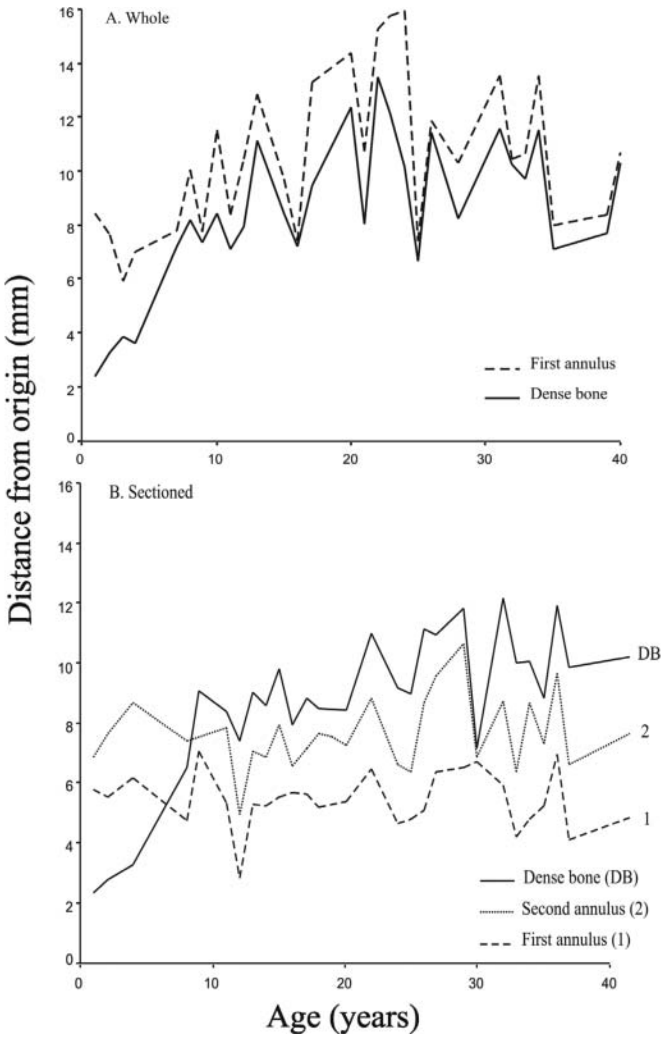

FIGURE 2. Comparison of aging techniques for longnose sucker opercula: **(A)** the distance from origin to distal edge of dense bone compared with distance from origin to first annulus for whole operculum technique, versus **(B)** distance from origin to first and second annuli in sectioned operculum technique. Distance measures were taken from trial 1 of both aging techniques.

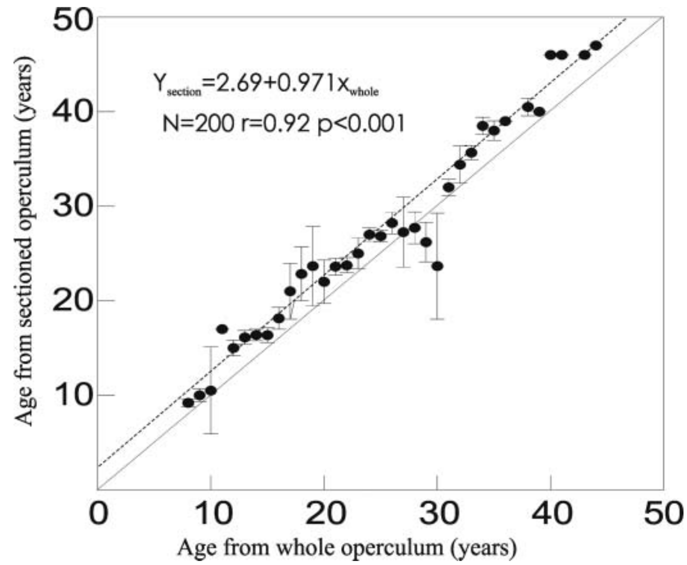

FIGURE 3. Age bias plot comparing longnose sucker ages assigned by two age interpretation techniques. Error bars represent the 95% confidence interval around the mean age assigned using the sectioning technique for all opercula assigned a given age using the whole operculum technique. The solid line represents the 1:1 relationship between techniques. The dashed line represents the regression of the plotted ages assigned by the two age interpretation techniques.

sectioning technique for both the reproducibility of age estimates and location of the first five annuli (Table 1).

The paired *t*-test revealed a significant effect for trial mean fish age between techniques (Table 2). When looking at the average for the entire sample, interpreted ages from the whole operculum technique were 1 year younger than the interpreted ages for the sectioning technique. When samples were compared based on age subgroups, we found only a slight assigned age difference (0.13 years) between techniques for young fish (age $\leq$6) and a 2-year assigned age difference for the older subgroup (age $\geq$10; Table 2).

The position of the distal edge of dense bone relative to the interpreted position of the first annulus (Figure 2A) indicated an influence of overlying dense bone on age interpretation when using the whole-operculum technique. The first and second annuli that were obscured by dense bone, and undetected in the whole operculum technique, were revealed using the sectioning technique (Figures 1C, 2B). We were unable to delineate the progression of dense bone growth through fish age 6–10 because of small sample sizes. The age-bias plot also revealed a trend towards older ages with the sectioning technique (Figure 3).

TABLE 1. Paired *t*-tests comparing the repeatability of age determinations for longnose suckers between whole and thin-sectioned operculum techniques (1) using mean CVs ($N = 95$ pairs) and 95% confidence intervals (CIs) calculated from the four aging trials, and (2) the precision of annulus measurement between techniques for the first five annuli and the last five annuli. Coefficient of variation values were calculated for each of the 10 annulus positions (per operculum) using measurements taken during the four trials, and were averaged for the first five annuli and last five annuli and compared between techniques ($N = 47$ pairs). Means and 95% confidence intervals (CI) were calculated using individual CV values (only fish age 11 and older were included in the analysis).

Comparison	Whole-operculum mean (CI)	Thin-sectioning mean (CI)	*t*	*P*
Repeatability of age (1)	3.50 (2.31–4.69)	8.03 (5.77–10.29)	−3.37	< 0.001
First five annuli (2)	7.81 (6.19–9.42)	15.88 (13.56–18.21)	−8.07	< 0.001
Last five annuli (2)	2.90 (1.25–4.56)	4.64 (2.41–6.87)	−1.24	0.222

TABLE 2. Paired t-tests comparing mean longnose sucker age differences for the entire sample and for age subgroups. Mean age estimates and 95% confidence intervals (CIs) were calculated using the four age interpretation trials of the whole-operculum and thin-sectioning techniques.

Age grouping	Whole-operculum mean (CI)	Thin-sectioning mean (CI)	t	P
All ages combined	12.10 (10.91–13.29)	13.13 (11.83–14.43)	−7.266	$p < 0.001$
Age 6 and younger	1.37 (1.22–1.52)	1.24 (1.13–1.36)	2.715	$p = 0.007$
Age 10 and older	22.17 (21.03–23.13)	24.32 (23.13–25.51)	−8.626	$p < 0.001$

DISCUSSION

We hypothesized that due to an overgrowth of dense bone near the hyomandibular socket, an age bias would exist where whole-operculum interpretations would be of a younger age than sectioned opercula; we thought that the underlying zones could be detected and measured as precisely as zones in the whole operculum. Overall, the whole-operculum technique underestimated age by 1 year. When we further split the samples into subgroups by age, there were no differences between techniques for young fish (age $\leq$6; a difference of 0.13 years), but there was a 2-year difference (sectioned being consistently greater) when we compared techniques for older fish (age $\geq$10).

Measurements of dense bone overgrowth from samples prepared with the sectioning technique compared with distances from the origin to the first and second annuli of older fish showed that the first few annuli were obscured under dense bone. However, the lack of an age difference between techniques for the young subgroup suggests that dense bone overgrowth does not begin to obscure annuli until the ages of 6–10. Thus, when using the whole operculum technique, interpreters should be aware that as fish become older, increments and annuli located close to the origin become obscured. Further, the bias associated with dense bone overgrowth seems to be consistent throughout the age span of older longnose suckers (age $\geq$10). The dashed line in Figure 3 runs parallel to the 45° construction line. This is different from the frequently demonstrated relationship in age bias plots for scale verses otolith age in that as the fish gets older, the line does not diverge in a progressively greater degree (Campana et al. 1995). Therefore, it would appear that bias created by dense bone is limited. Others have had similar findings. When aging the catostomid cui-ui, Scoppettone (1988) discovered that the fenestrated reinforcement bone associated with the hyomandibular socket covers annuli, and depending on the age of the fish being examined, between 0 and 3 annuli could be hidden; however, fish younger than 6 years had no hidden annuli.

Others who have attempted to age fish from whole opercula have found a similar problem. Marcogliese (1996) found that back-calculations of white sucker *Catastomus commersonii* growth from opercula revealed an absence of earlier annuli from interpreted ages for fish age 8 and older. Northern pike greater than age 7 had at least one or two annuli missing (Frost and Kipling 1959). In our study, the sample sizes for ages 7–9 were very small; therefore we were unable to determine the exact age at which individual annuli were first affected by dense bone. More sampling is needed to determine this age.

For both techniques, the mean coefficient of variation values and confidence intervals around the mean estimates were very small, indicating the utility of both techniques for the repeatability of age determination and delineation of annuli. Nevertheless, contrary to our second hypothesis, reduced bone density, created by thin-sectioning of the operculum, did not result in more precise location determinations than in whole opercula. Thin-sectioning resulted in less interpretive repeatability of fish age and annuli location than for the whole-operculum technique. The variation associated with age assessment using the thin-sectioning technique was more than twice that of the whole operculum technique. Increased variance associated with the sectioning technique may result from difficulty in distinguishing and tracking zonation as extensively as can be done on the whole operculum. We found that when interpreting operculum sections, it was difficult to discern between individual annuli because transitions between translucent and opaque zones were more gradual. If an interpreter is attempting to identify indistinct annuli, the ability to relocate the exact point at which an annulus occurs is reduced (Braaten et al. 1999). Thus, greater care must be taken when making interpretations using the sectioning technique. Some of the variance in age interpretation and annulus identification can be reduced with the thin-sectioning technique during sample preparation. We learned that a critical step during preparation of the thin section was ensuring that the cut of the operculum was made perpendicular to the plane of the zones. If the section was cut at an angle greater or less than 90°, the transition between translucent and opaque became obscured.

A disadvantage of the sectioning method is preparation time. Total preparation time for the 106 samples was approximately 7 d (2 d to embed and harden, 3 d to section and 2 d to mount and polish). Further, the technique takes some practice to do correctly. Several of our samples were destroyed due to breakage, poor sectioning, or overpolishing. Therefore, we recommend that before undertaking this method, a subset of samples should be used to perfect the technique.

The bias of underestimating age when examining whole opercula could lead to greater uncertainty in our predictive abilities as fisheries managers. Effective recruitment models are based on accurate age estimates. Predictions of both growth rates and mortality rates may be inadvertently affected if age is misrepresented. Therefore, it is important that fisheries managers achieve both precision and accuracy when interpreting age.

Some researchers used the Ford–Walford plot (Marcogliese 1996) to back-calculate age, compensating for the absence of early annuli. However, theoretical annuli locations calculated from the Ford–Walford plot may inadvertently eliminate important growth information, leading to misinterpretation of growth changes. Therefore, when reconstructing growth from opercula from old, slow-growing suckers, sectioning would be a more reliable technique.

Determining age from whole bones, expecially in older fish, could result in an underestimation bias. Where overgrowth occurs or is suspected, sectioning should be conducted to better examine and quantify the problem. This technique may have the potential to be generalized to other flat bones and long-lived species.

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