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Precision of Estimated Ages of Lake Trout from Five Calcified Structures

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Abstract.—Precisions of estimated ages from vertebrae, cleithra, opercular bones, otoliths, and scales were measured in replicated trials on samples from lake trout *Salvelinus namaycush* from interior Alaska. Ages from all structures were similar for sexually immature lake trout. For mature lake trout, estimated ages from otoliths and from opercular bones were the most precise, although ages from opercular bones were significantly younger by a year than ages from otoliths. Estimated ages from cleithra and whole vertebrae were, respectively, too imprecise and too low for these structures to be useful in age validation studies. On this basis, otoliths and opercular bones can be used in age validation studies and scales can be used to estimate the age of immature lake trout.

Although scales have been traditionally used to estimate the age of lake trout *Salvelinus namaycush* (see Carlander 1969), ages estimated from scales have differed from ages estimated from other body parts, particularly branchiostegal rays (Bulkley 1960) and otoliths (Dubois and Lagueux 1968; Johnson 1976; Power 1978; Martin and Olver 1980). Counts of annuli on all structures from immature fish were similar, but fewer annuli were counted on scales than on other structures from larger fish. This divergence in estimates begins when scale growth slows relative to body growth while other structures, especially otoliths, continue growing (Dubois and Lagueux 1968; Simard and Magnin 1972; Johnson 1976).

The difficulty in determining the ages of lake trout with scales, otoliths, or any other body structure is a lack of reliable criteria from validation studies (see Beamish and McFarlane 1983). Such studies are now beginning in Alaska, and scales and otoliths are being collected. However, before ages from otoliths, scales, or any other structure can be validated, they must be precisely estimated. An accurate but imprecise estimate of age is worthless. A series of replicated analyses was conducted on a sample of lake trout from interior Alaska as a prelude to validation studies on this species. Variation in estimated ages from scales, otoliths, cleithra, vertebrae, and opercular bones was analyzed, along with variation due to readers and variation in the time taken to make these estimates.

Methods

During September 1986, 90 lake trout were collected with gill nets from Summit Lake (63°07′45″N, 145°31′40″W), site of a major sport fishery for lake trout in interior Alaska. Otoliths (sagittas) were collected from all fish, and scales, cleithra, thoracic vertebrae, and opercular bones were collected from 25. These calcified structures were chosen because they have been used to determine the ages of lake trout and other species (see Beamish and McFarlane 1987). Scales were sampled from a location immediately above the lateral line below the posterior base of the dorsal fin on each fish. They were mounted on slides and examined with a microfiche reader. Criteria for determination of age from scales were taken from Cable (1956). Whole otoliths were hand-polished along the sagittal plane on a carborundum honing stone, washed in water, and placed in a mixture of glycerine, alcohol, and water for viewing with a dissecting microscope at moderate magnification (12× or 25×) under reflected light. Each translucent band was considered an annulus, and annuli were counted from the kernel outward. The direction of counting was the direction that had the largest count. Thoracic vertebrae were removed from the spine at a location directly below the posterior margin of the dorsal fin. They were brushed free of all muscle and connective tissue, washed with soap and hot water, and air dried. Whole vertebral centra were examined under a dissecting microscope at low magnification (6×) with reflected light. Counting began at the focus of the centrum and proceeded out to the edge; each concentric, calcified ridge was considered an annulus. Cleithra and opercular bones were cleaned as were vertebrae, then examined under a dissecting microscope at low magnification with re-

TABLE 1.—Mean counts of annuli in lake trout structures by three readers in the structure study. Each reader mean was calculated from 75 observations (e.g., 25 otoliths from 25 lake trout, each otolith read 3 times).

Statistic	Vertebrae	Scales	Opercular bones	Cleithra	Otoliths
Reader means					
Reader 1	7.95	8.68	8.92	10.87	9.80
Reader 2	6.16	6.84	8.32	10.21	8.95
Reader 3	8.84	7.65	8.21	7.09	9.55
Average of readers	7.65	7.72	8.48	9.39	9.43
Maximum difference among reader means	2.68	1.84	0.71	3.77	0.85

flected light against a black background. Translucent bands were considered annuli on both cleithra and opercular bones. When these translucent bands were indistinct against the black background, the structure was held up against a source of transmitted light for reading as suggested in other studies (Le Cren 1947; Casselman 1974). For opercular bones, counting began with the first distinct translucent band out from the posterior rim of the fulcrum and proceeded diagonally towards the posterior margin of the bone as recommended by McConnell (1952). For cleithra, counting began from the origin out towards the ventral edge of the anterior blade as recommended by Casselman (1974).

Comparison of structures.—The first part of the study (hereafter called the structure study) was designed to measure the repeatability of estimated ages (hereafter called counts), the differences in counts among the five structures, and the expected differences among potential readers. Three persons (hereafter called readers) counted annuli on five structures from 25 lake trout three times each (3 readers × 25 fish × 5 structures × 3 replications = 1,125 readings). The test fish were 18 males and 7 females with fork lengths from 231 to 691 mm. Readers had equal experience working with scales and otoliths prior to this study, less experience with vertebrae, even less with cleithra, and no experience working with opercular bones. Each reader examined the same structure from all fish and then proceeded to the next structure until all structures were examined. The order of examination was randomly selected for each reader. All structures were consecutively numbered, and the first structure to be examined was randomly selected; subsequent examination proceeded in order. Each reader recorded the time required to read each set of 25 structures. Readers were unaware of the lengths of the lake trout in the study.

The sampling standard error was chosen as an index of the repeatability of counts for the structure study. Differences in repeated counts from the same structure, from the same fish, and by the same reader are "mistakes." Those structures that are associated with the largest differences (the biggest mistakes) are the least desirable for use in determining the age of lake trout. The sampling variance for each structure–fish combination is the mean squared error between counts (X_{ij}) and mean counts ($\bar{X}$) repeated (n times) and averaged across readers (r):

$$V(\bar{X}) = \frac{\sum_{i}^{r} \sum_{j}^{n} (X_{ij} - \bar{X}_i)^2}{r(n-1)}.$$

The sampling standard error (the square root of the sampling variance) is analogous to the average percent error used by Beamish and Fournier (1981) and to the coefficient of variation proposed by Chang (1982), except that sampling standard error is not scaled to the age of the fish as are these other two indices.

Comparison of readers.—The second analysis (hereafter called the reader study) was designed to more precisely measure the expected differences in counts from otoliths among readers. The procedures for this analysis were the same as those in the structure study except three more readers and otoliths from 25 more lake trout were added to make 6 readers, 50 otoliths, and 3 replications (6 × 50 × 3 = 900 readings). The additional fish consisted of 8 males, 14 females, and 3 of unknown sex; fork lengths ranged from 126 to 872 mm.

Results

Comparison of Structures

Mean counts varied considerably among structures and among readers for each structure (Table 1). Averages of mean counts from scales and vertebrae were similar (7.7 years) as were averages for otoliths and cleithra (9.4 years); averages of

TABLE 2.—Standard deviations of annulus counts on lake trout structures by three readers in the structure study. Each SD was calculated from 75 observations (e.g., 25 vertebrae from 25 lake trout, each vertebra read three times).

Statistic	Vertebrae	Scales	Opercular bones	Cleithra	Otoliths
Reader SDs					
Reader 1	4.37	3.77	5.46	5.73	6.52
Reader 2	4.01	3.75	5.55	5.38	6.34
Reader 3	5.59	2.95	5.62	4.65	5.40
Average of reader SDs	4.66	3.49	5.54	5.25	6.09
Maximum difference among reader SDs	1.58	0.82	0.16	1.08	1.12

mean counts from opercular bones were intermediate. Differences among readers in mean counts were largest for cleithra (3.77 years) and smallest for opercular bones and otoliths (0.71 and 0.85 years, respectively). Variation among replicate counts made by individual readers was greatest for otoliths (SD = 6.09 years) and smallest for scales (SD = 3.49 years; Table 2); the standard deviations varied across readers from 1.58 years for vertebrae to 0.16 years for opercular bones.

Much of the variation among counts of annuli from cleithra was due to the inability of readers to consistently repeat their counts over replicated readings (Figure 1). Sampling standard errors were less than 2 years for most fish and for all structures except cleithra. Sampling standard errors for cleithra were 3 years or less for most fish, but exceeded 5 years for one lake trout. For scales, otoliths, opercular bones, and vertebrae, sampling SEs were higher for older, larger fish than for younger, smaller lake trout; for cleithra, sampling SEs were large even for small fish. Because of the large measurement errors in the replicated counts from cleithra, and because of the large variation in mean counts among readers for this structure (Table 1), cleithra were disregarded in further analyses.

Differences in mean counts among structures were greater for larger, older fish, especially for mature lake trout, i.e., fish larger than 400 mm fork length (Burr 1987; Figure 2). For lake trout less than 400 mm, the average range in mean counts for the four structures was 1.62 years; for larger fish the average range was 5.76 years. Counts from scales were markedly skewed toward younger ages compared to counts from the other three structures (Figure 3). Distributions of counts from opercular bones, vertebrae, and otoliths were similar, except that the largest counts (oldest estimated ages) were obtained from otoliths.

An analysis of variance (ANOVA) of otolith and opercular bone counts showed that the mean estimated age from otoliths (9.43 years) was significantly larger ($P < 0.05$) than that from opercular bones (8.48 years) by an expected 0.95 years. The ANOVA was based on the procedures of general linear models for a balanced design with structures as fixed effects, readers as random effects, and fish as an exogenous source of variation. The expected difference among readers was a significant 0.73 years ($P < 0.05$) with the three readers divided into two groups based on multiple comparisons of least significant differences ($P = 0.05$). Mean

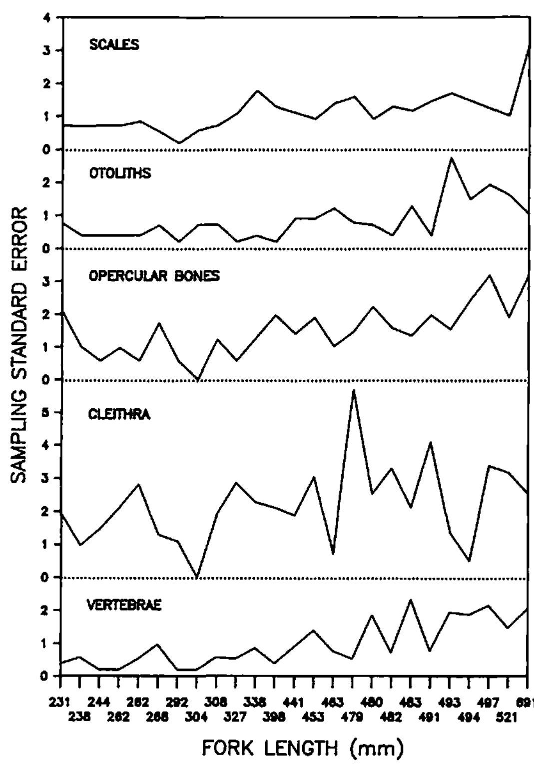

FIGURE 1.—Sampling standard errors of annulus counts, by structure, for all lake trout used in the structure study and studied by three readers. Units on the abscissa are individual fish in order by length. Each point corresponds to 6 df.

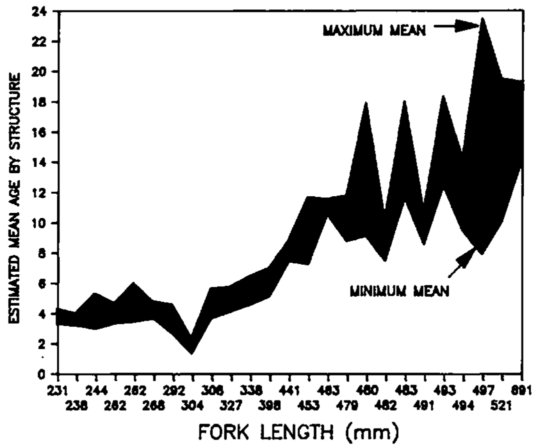

FIGURE 2.—Ranges of mean annulus counts among scales, otoliths, opercular bones, and vertebrae for each lake trout used in the structure study. Mean counts for each fish by structure were averaged across readers and replicates (nine readings per mean). Units on the abscissa are individual fish in order by length.

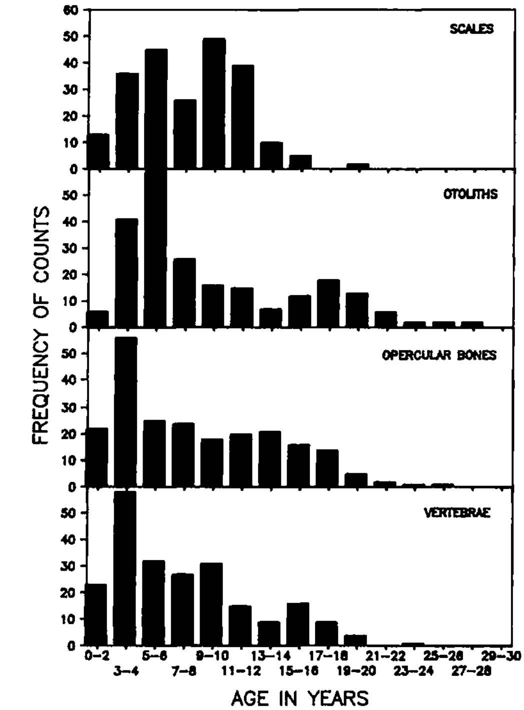

FIGURE 3.—Age-frequency distributions for annulus counts on four structures from 25 lake trout by three readers with three replicates each (each histogram represents 225 readings).

counts from scales and from vertebrae were excluded from this ANOVA because their overall means were similar (7.65 years for vertebrae and 7.72 years for scales), and estimated ages from scales are known to be too low for mature lake trout in arctic and subarctic regions (Johnson 1976).

Comparison of Readers

Mean counts of annuli in otoliths from the 50 lake trout used in the reader study ranged from 9.89 to 10.67 years for the six readers. An ANOVA, based again on a general linear model of a balanced design with readers as random effects and fish as an exogenous source of variation, showed that the differences in mean counts among readers were significant ($P < 0.01$); the expected difference in mean counts among readers was 0.78 years (in contrast to the 0.73 years in the structure study). A multiple comparison test based on least significant differences ($P = 0.05$) showed that readers were divided into two groups of four and two readers each.

Comparison of Reading Times

In the structure study, otoliths and scales took longer to read than did opercular bones and vertebrae. The first, second, and third replications of five structures by three readers took 902, 591, and 502 min, respectively. Because the readers obviously became more proficient as they gained experience, only the third replication was considered representative of the time needed to read each set of structures. The readers finished all vertebrae and opercular bones in averages of 1.55 and 1.4 h, respectively; otoliths and scales were read in 1.98 and 1.83 h, respectively. However, these reading times were not significantly different among structures (Friedman Rank Sum: $S = 6$; $P > 0.22$). In the reader study, the average time across replications needed to obtain 75 counts (3 readers on 25 otoliths) was 1.96 h (the comparable statistic for the third replication in the structure study was 1.98 h).

Discussion

Of the five calcified structures used in this study, only opercular bones appeared to be an attractive alternative to otoliths as a means of determining the age of lake trout. Readers could not repeat their annulus counts from cleithra consistently; estimated mean age of the sample from vertebrae was similar to that from scales and thus was too low. Although mean age of the sample estimated from opercular bones was a year younger than the estimate from otoliths, the precisions of these estimates were similar. The best agreements on the average age of the sampled lake trout among readers occurred for opercular bones and for otoliths. This was surprising, because readers had experience with otoliths and no experience with opercular bones prior to this study.

The standard deviation of counts was slightly larger for otoliths than for opercular bones, as would be expected for a population with a slightly larger mean. However, the range of SDs among readers was much smaller for opercular bones than for otoliths. The difference of a year in estimated mean ages from otoliths and from opercular bones most likely arose because readers missed the first annulus in many or all of the opercular bones. Extension of the posterior rim of the fulcrum obscures the first annulus on opercular bones from other teleosts (Le Cren 1947; McConnell 1952; Neuhold 1956; Frost and Kipling 1959). The extent that this phenomenon occurred in our samples was unknown. Analysis of the growth of hatchery-reared lake trout, as was done by Cable (1956), should clarify the magnitude of this problem with opercular bones.

Although the results of this study show, as do others (Dubois and Lagueux 1968; Simard and Magnin 1972), that estimated ages from scales are lower than estimated ages from otoliths, scales can still be useful in determining the age of immature fish. Estimated ages from scales taken from immature lake trout are generally based on true annuli (Cable 1956; Fry and Budd 1958). Estimated ages from scales were similar to those from other structures for immature lake trout in this study. Scales also are the only structures of those studied that can be sampled repetitively from the same fish during long-term age validation studies.

Inspection of whole vertebrae, as was done in this study, has led to accurate estimates of age for some teleosts (Appelget and Smith 1951; Zubrock 1952), but not for others (Prince et al. 1985). The mean age of lake trout estimated from whole vertebrae in our study was almost identical to the estimate from scales, and thus too low. Low estimates of age from scales are due to losses of the annual scale deposition of minerals from abrasion (Alvord 1954; Linfield 1974) or to a marked reduction in that deposition in slow-growing fish (Johnson 1976). Because the growth of lake trout slows markedly after maturation, the age frequencies of a population determined from scales would be trunated, as was the case in this study. However, the age-frequency distribution for whole vertebrae was not truncated; rather, it declined gradually with age as did the frequencies of ages estimated from opercular bones and otoliths. Unlike the missing annuli on scales, all annuli were probably present in the samples of vertebrae, but were difficult to detect, as was the case with Atlantic tuna *Thunnus thynnus* (Prince et al. 1985). The relatively large variation in mean ages among readers also indicated that annuli were difficult to detect on vertebrae. If annuli are more detectable on sectioned vertebrae, this structure still has promise as a means of accurately determining the age of lake trout.

The slow growth of lake trout was most likely the indirect cause of the poor repeatability of counts from their cleithra. Translucent bands on the cleithra used in this study were more visible in transmitted light than in reflected light, which is consistent with cleithra from slower-growing fish (Casselman 1974). Cleithra from slower-growing esocids contain many translucent, pseudoannuli that complicate identification of true annuli (Casselman 1974; Harrison and Hadley 1979). Many such incomplete and indistinct translucent bands were found on samples from the lake trout used in this study. These samples came from a population of relatively fast-growing lake trout (Burr 1987), so it is likely that poor repeatability of counts from cleithra will be a problem with all arctic and subarctic populations of this species.

Variation in the ability of readers in this study undoubtedly affected the variation in estimates of mean ages, as has been noted elsewhere for estimated scale ages of lake trout (Martin 1966). The range of mean counts among readers was about the same in the structure study as in the reader study (0.73 and 0.78 years, respectively). This range of about three-quarters of a year was similar to the range of mean counts among readers in the structure study for opercular bones (0.71 years) and for otoliths (0.85 years). Therefore, the exhibited variation in the estimated mean ages from otoliths and opercular bones was mostly a reflection of the differences in the abilities of individual readers. In contrast, the differences of estimated mean ages from vertebrae and scales among readers (2.68 and 1.84 years, respectively) were considerably larger than the underlying variation among readers (0.73 years). This reader–structure interaction was not reflected in the time needed to read the structures. Readings of otoliths and scales were completed in about 2 h; readings of opercular bones and vertebrae were completed in about 1.5 h. Reading time was not a reflection of the experience of our readers. Although our readers were quicker at reading opercular bones than otoliths (the difference was not significant), they were more experienced with otoliths.

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