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PRECISION OF ESTIMATED AGES OF BURBOT USING VERTEBRAE AND OTOLITHS[1]

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ABSTRACT

The precision of repeated age estimates made by four readers using vertebrae and otoliths collected from 156 burbot, *Lota lota* was examined using analysis of variance and the indices of standard deviation, average percent error, and coefficient of variation. Age estimates were concluded to be relatively precise within and among structures. Both vertebrae and otoliths were determined to be suitable candidates for age validation studies.

Key words: Burbot, *Lota lota*, vertebrae, otoliths, age estimates, age validation, and precision of ages.

INTRODUCTION

Burbot *Lota lota* are found throughout the Tanana River drainage in interior Alaska. Annual harvest levels of burbot in this area of Alaska have more than doubled in recent years. Given the increased pressure on burbot as a sport fish, a data base including age, length, weight, sex, and general distribution was initiated by the Alaska Department of Fish and Game (ADFG) in 1983 to assess the status of burbot stocks in the Tanana River.

Estimating age and growth can sometimes be accomplished through the study of length-frequency distributions, or by using tagging and recapture methods, but the most practical method is studying annuli laid down in bony structures of fish of known length (Chen 1969). While the use of bony structures for age estimation is common, questions of accuracy and precision remain. The question of accuracy must be addressed by validation, which has become a more frequent research topic in recent years (Beamish and McFarlane 1983). Validation is important if estimated ages are to be considered estimates of true age, but it is a wasted effort if the structures used give imprecise age estimates.

The scale is the most popular bony structure for estimating the age of fish (Everhart and Youngs 1981), but burbot scales are very small, deeply embedded, and often lack annuli. McCrimmon and Devitt (1954) used pectoral fin rays, scales, and otoliths for age determinations of burbot from Lake Simcoe, Ontario. They reported unsatisfactory results using scales, difficulties with younger age groups using fin rays, and had the best results using otoliths. Martin (1941) used otoliths for age determinations of burbot, citing the similarity of burbot otoliths to cod *Gadus callarias* otoliths, for which the use of this structure for age determination was well established. Since then, otoliths have been used frequently for age determinations of burbot (e.g. Chen 1969; Clemens 1951; Hewson 1955; Bailey 1972).

Vertebrae, like otoliths, require that fish be killed and dissected to obtain the aging structures, but they have not been used as extensively as otoliths. Chatwin (1956) used vertebrae for age estimates of lingcod *Ophiodon elongatus*. He concluded that the structure gave reliable estimates, but that they are not practical for commercially caught fish due to the time required for processing the structures and the damage to the fish carcass caused during sampling. Clark (1987) compared vertebrae, otoliths, and scales for use in aging fall chum salmon *Oncorhynchus keta*. He concluded that the time required to process and read vertebrae (twenty times as long as scales) made them less practical to use but that the precision and accuracy involved with vertebrae made them the best of the three structures researched. Though vertebrae have practical limitations, they seem to yield reliable age estimates.

Based on reports in the literature, vertebrae and otoliths were chosen as test structures for estimating ages of burbot in our study. Do the structures give significantly different age estimates? Does one structure give more repeatable age estimates than the other? Are there significant differences in age estimates between readers? Are vertebrae and otoliths suitable candidates for validation studies? In this study we address the precision of age estimates made using vertebrae and otoliths from burbot, and make recommendations regarding the use of these structures in future validation studies.

METHODS

Sampling

Using a large outboard-powered riverboat, hoop nets were set in the Tanana River in locations selected according to fluctuating water levels. Netting took place during summer and fall months of 1984, 1985, and 1986. The hoop nets were attached to the shore with the opening facing downstream, and they were baited with frozen Pacific herring *Clupea harengus* placed in perforated plastic containers located in the cod end of the hoop trap.

The otolith (sagitta) and a section from between the 3rd and 5th vertebrae containing at least two centrum bones were collected from each of the 156 burbot. Vertebrae were kept frozen until the end of the field season, when they were thawed, placed in solution of dish soap and water for approximately 24 hours, cleaned with a stiff-bristle tooth brush, and allowed to dry. The otoliths were cleaned, broken in cross section, and ground on a whetstone.

Four readers counted annuli on both structures from 156 fish three times (4 readers x 2 structures x 156 fish x 3 replicates = 3,744 readings). Structures were read against a black background under reflected light at magnifications ranging between 12x and 15x using a Nikon compound microscope. Otoliths were read in a liquid medium (Loess solution). Each reader read all 156 vertebrae in one replicate before beginning readings using otoliths. Reader experience varied (Table 1). Three readers had experience with burbot vertebrae, and one reader had no experience reading vertebrae. Since all readers had some experience in making age determinations by counting annuli they were not instructed in methods of counting annuli, and no criteria for all readers were established. Readers did not know the lengths or sex of any of the fish.

ANALYSIS

Sampling standard error was used as an index of repeatability of counts (Sharp and Bernard 1988). The sampling variance for each fish is the mean squared error of age estimates (X_{ij}) repeated n times and summed across r readers:

$$V[\bar{X}_k] = \frac{\sum_{i}^{r}\sum_{j}^{n}(X_{ijk}-\bar{X}_{jk})^2}{r(n-1)}; \qquad (1)$$

where: X_{ijk} = the jth replicate of the kth fish by reader i; and,

$\bar{X}_{ik}$ = the mean estimate by reader i.

Table 1. Experience of the four readers making age determinations with vertebrae and otoliths[a].

	Vertebrae	Otoliths
Reader 1	0	2
Reader 2	1	1, 2
Reader 3	1	1
Reader 4	1	1, 2

[a] 0 = no experience; 1 = experience with other species; 2 = experience with burbot.

The sampling standard error is the square root of Eq. 1, and is analogous to the average percent error (APE) of Beamish and Fournier (1981) and to the coefficient of variation (CV) proposed by Chang (1982). Unlike the latter two indices, sampling standard error is not standardized by the age of the fish.

We used the standard deviation (SD) of age estimates as a measure of precision for all reader-structure combinations:

$$SD_i = \frac{\sum_{k}^{N} \sum_{j}^{n} (X_{ijk} - \bar{X}_{jk})^2}{N(n-1)}. \qquad (2)$$

Due to the previous use of APE and CV by other authors, and because this facilitates comparisons between populations having different numbers of age groups (Beamish and Fournier 1981; Chang 1982), we include these indices for comparisons of precision among reader-structure combinations. APE was calculated as follows:

$$APE_i = \frac{\sum_{k}^{N} \sum_{j}^{n} \frac{(\bar{X}_{ijk} - X_{jk})}{\bar{X}_{jk}}}{Nr}; \qquad (3)$$

$$\text{where: } X_{jk} = \sum \frac{X_{ijk}}{n}.$$

To test the significance of differences in age estimates between structures and between readers, a balanced, three-way, mixed effects ANOVA (Neter et al. 1985) was conducted. F-tests were designed following criteria in Zar (1984). An F-test for homogeneity of variances among structures showed no significant differences ($p > 0.50$), and both structures were included in a single analysis of variance.

RESULTS

Analysis of variance showed no significant difference in age estimates between vertebrae and otoliths ($0.25 > p > 0.10$). Readers, however, did differ significantly in their age estimates ($p < 0.0005$). Reader-structure interactions were also significant ($0.01 > p > 0.005$).

Ages in the sample ranged from 3 to 20 years based on average estimates from both structures. (Figures 1 and 2). Mean age estimates (Table 2) showed little variation among structures and among readers within structures, though differences between readers were statistically significant based on the ANOVA. The mean age estimate for otoliths was only 0.25 years older than the estimate using vertebrae. The range of mean estimates among readers was less than one year (0.84 years) for vertebrae, and only slightly more than one year

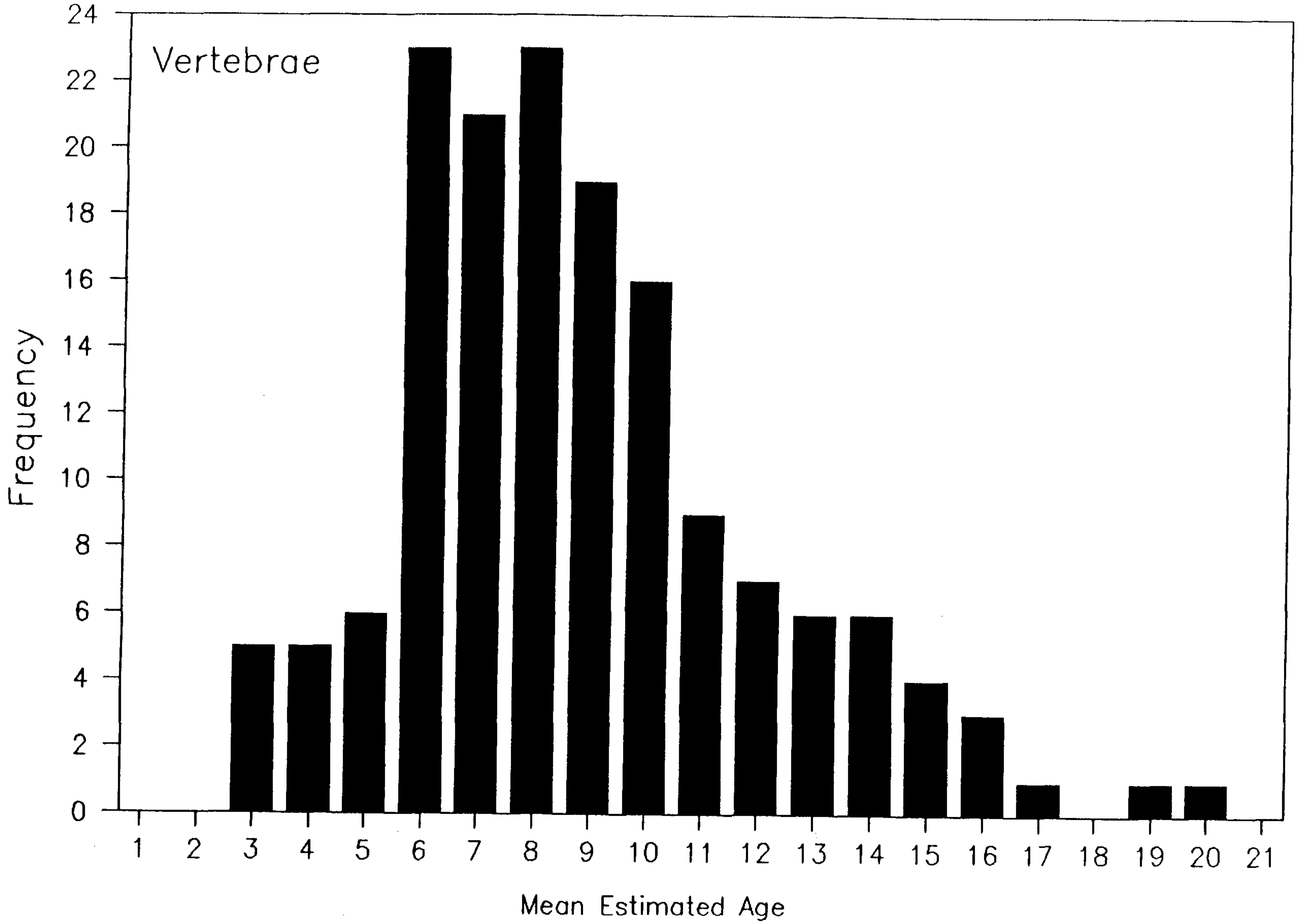

Figure 1. Mean estimated ages of 156 burbot based upon annuli counts of vertebrae.

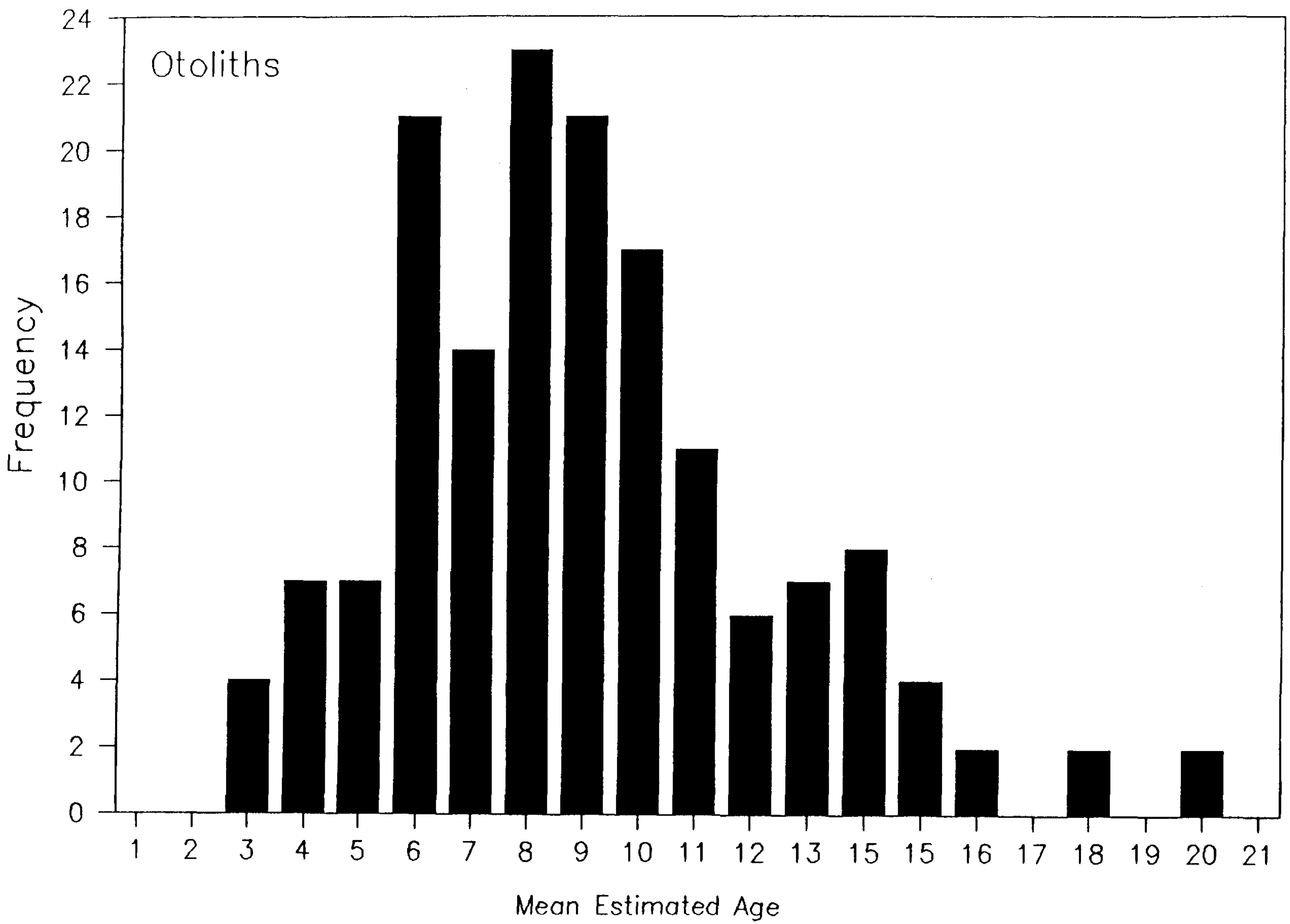

Figure 2. Mean estimated ages of 156 burbot based upon annuli counts of otoliths.

Table 2. Mean age estimates, standard deviations (SD), coefficient of variation (CV), and average percent error (APE) for all reader-structure combinations[a].

Reader	Vertebrae				Otoliths			
	Mean	SD	CV	APE	Mean	SD	CV	APE
1	9.26	1.2	0.13	4.2	9.56	0.4	0.05	3.7
2	9.64	0.7	0.07	5.6	10.02	0.8	0.07	6.1
3	9.15	0.2	0.03	2.1	9.31	0.4	0.04	3.6
4	8.80	0.4	0.05	4.1	8.93	0.5	0.05	4.0
Mean	9.21	0.6	0.07	4.0	9.46	0.5	0.05	4.3
Range	0.84	1.0	0.10	3.5	1.09	0.4	0.03	2.5

[a] Calculated from 468 observations (156 burbot x 3 replicate readings).

(1.09 years) for otoliths. Ninety percent of the differences between vertebrae and otolith mean age estimates for the 156 individual fish differed by one year or less (Figure 3).

The standard deviation (SD_i) and coefficient of variation (CV_i) were highest for vertebrae when the reader had no experience (Table 2; Figures 4 and 5). Average percent error (APE_i) (Table 2; Figure 6) of reader 2 (one of the two most experienced readers) was greatest using both structures. Sampling standard error appears to increase as fork length increases for both otoliths and vertebrae (Figures 7 and 8).

DISCUSSION

The difference in mean age estimates between the two structures was 0.25 years, and ninety percent of the mean estimates for structure-fish combinations differed by one or less years. The results of the analysis of variance suggest that differences between structures are not significant. Burbot are grouped into one year-age classes, so differences of less than one year do not seem practically significant. The variances of the two structures are not significantly different. Based on these results, neither structure can be considered superior or recommended over the other. That is, both vertebrae and otoliths yielded similar age estimates and both were similarly precise.

SD_i and CV_i follow a similar pattern among readers. APE_i did not correspond with the patterns of SD_i and CV_i. None of the indices could be associated with patterns of mean age estimates among structures or reader experience. Based on ANOVA results, differences in age estimates between readers were significant. Reader experience varied considerably, though readers with more experience did not tend to be more precise. Reader three, with no prior experience with either structure on burbot, was the most precise for both structures. Reader two, one of the two most experienced with burbot otoliths, demonstrated the least precision with this structure. Evidently, reader experience is not necessarily a major factor associated with precision of estimates when using otoliths and vertebrae to age burbot.

Vertebrae and otoliths yielded similar age estimates and differed little in regard to precision of those estimates. Both structures require that fish be sacrificed. We believe that both vertebrae and otoliths are suitable bony structures for validation studies involving burbot.

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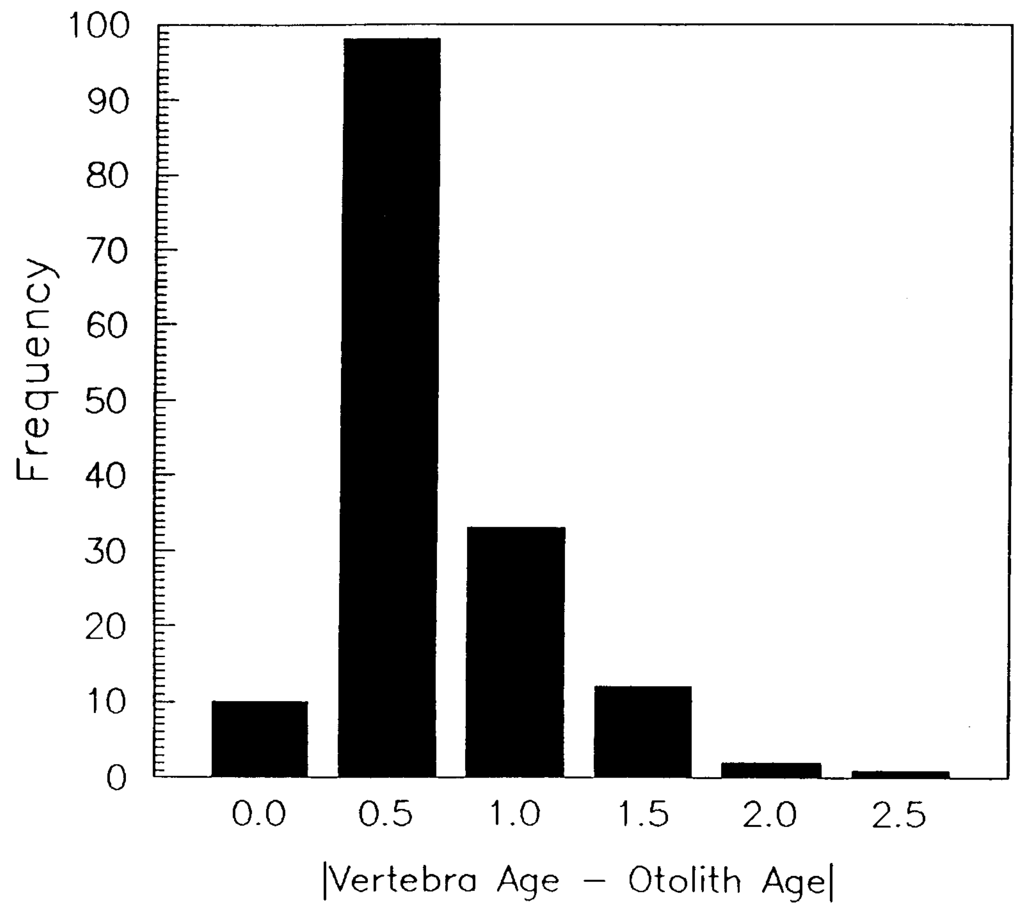

Figure 3. Distribution of the differences in estimated ages obtained through annuli counts of vertebrae versus otoliths for 156 burbot.

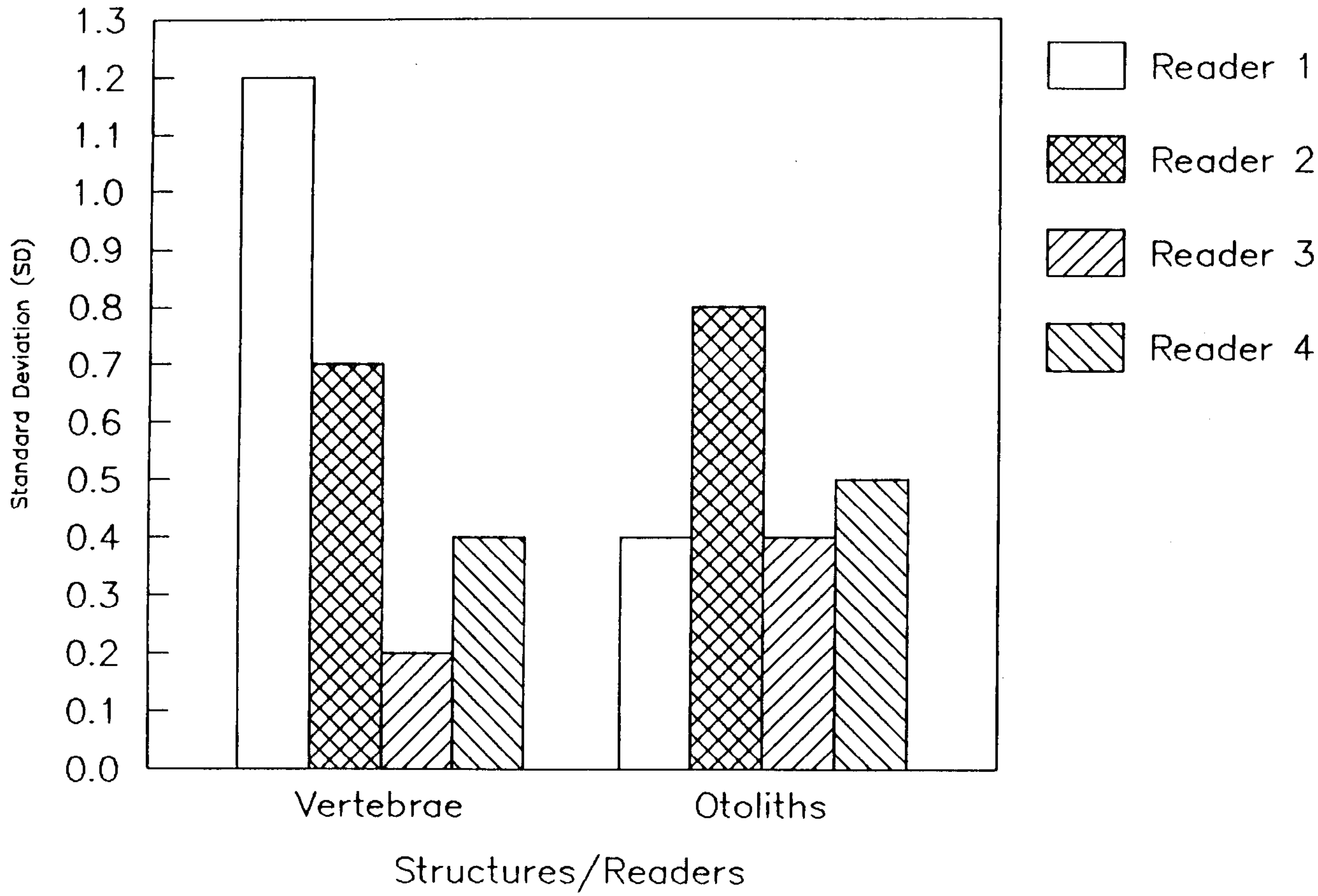

Figure 4. Standard deviations of replicate counts of annuli by four readers for vertebrae and otoliths.

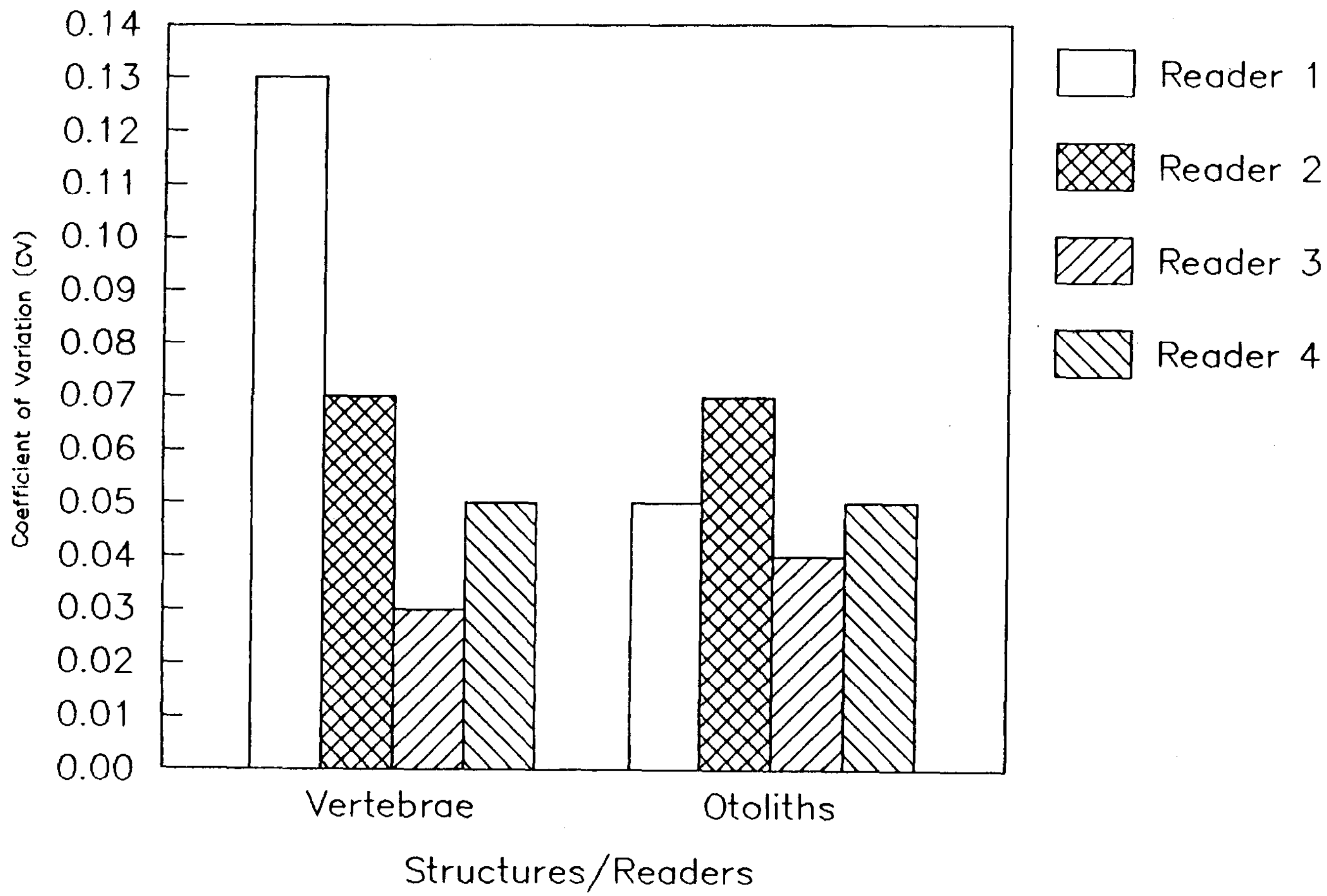

Figure 5. Coefficient of variation of replicate counts of annuli by four readers for vertebrae and otoliths.

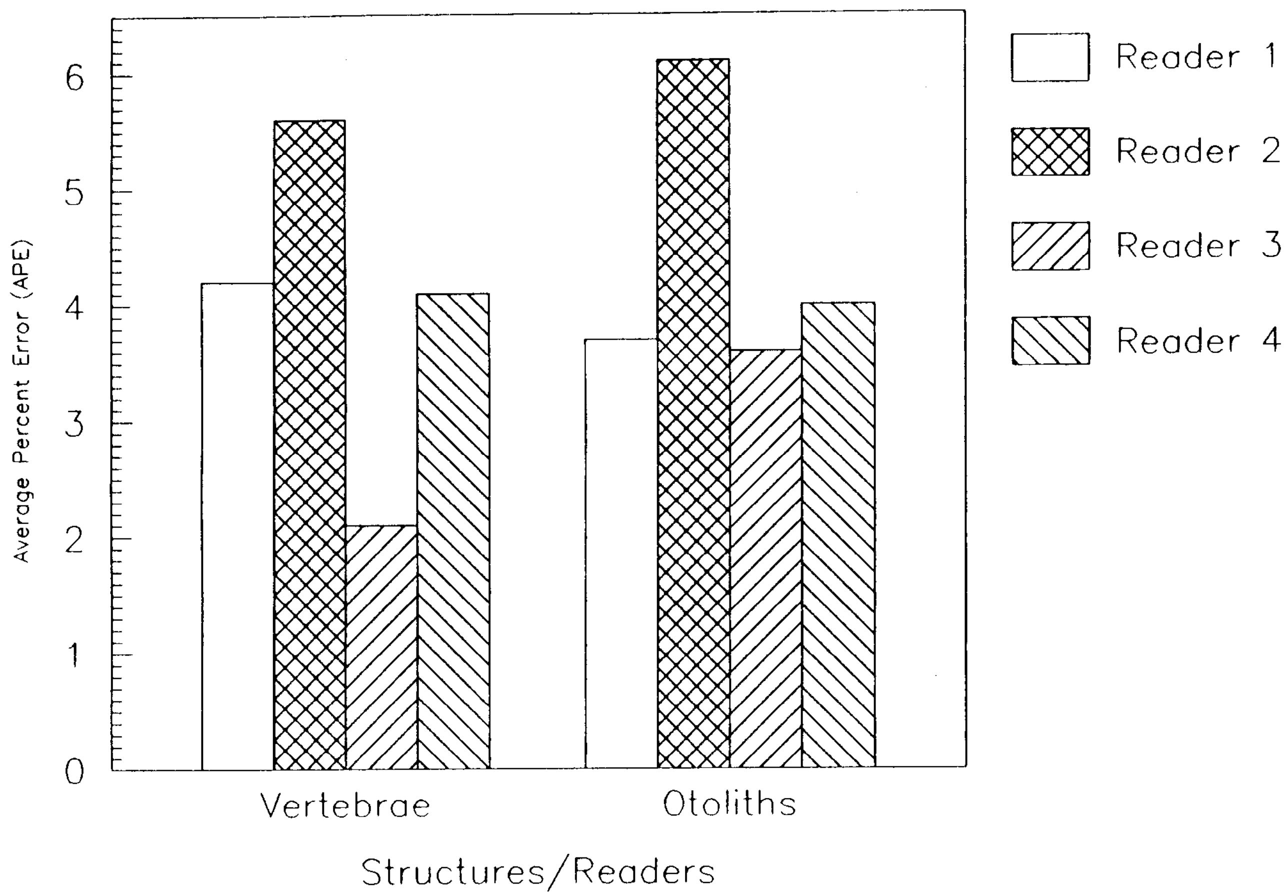

Figure 6. Average percent error of replicate counts of annuli by four readers for vertebrae and otoliths.

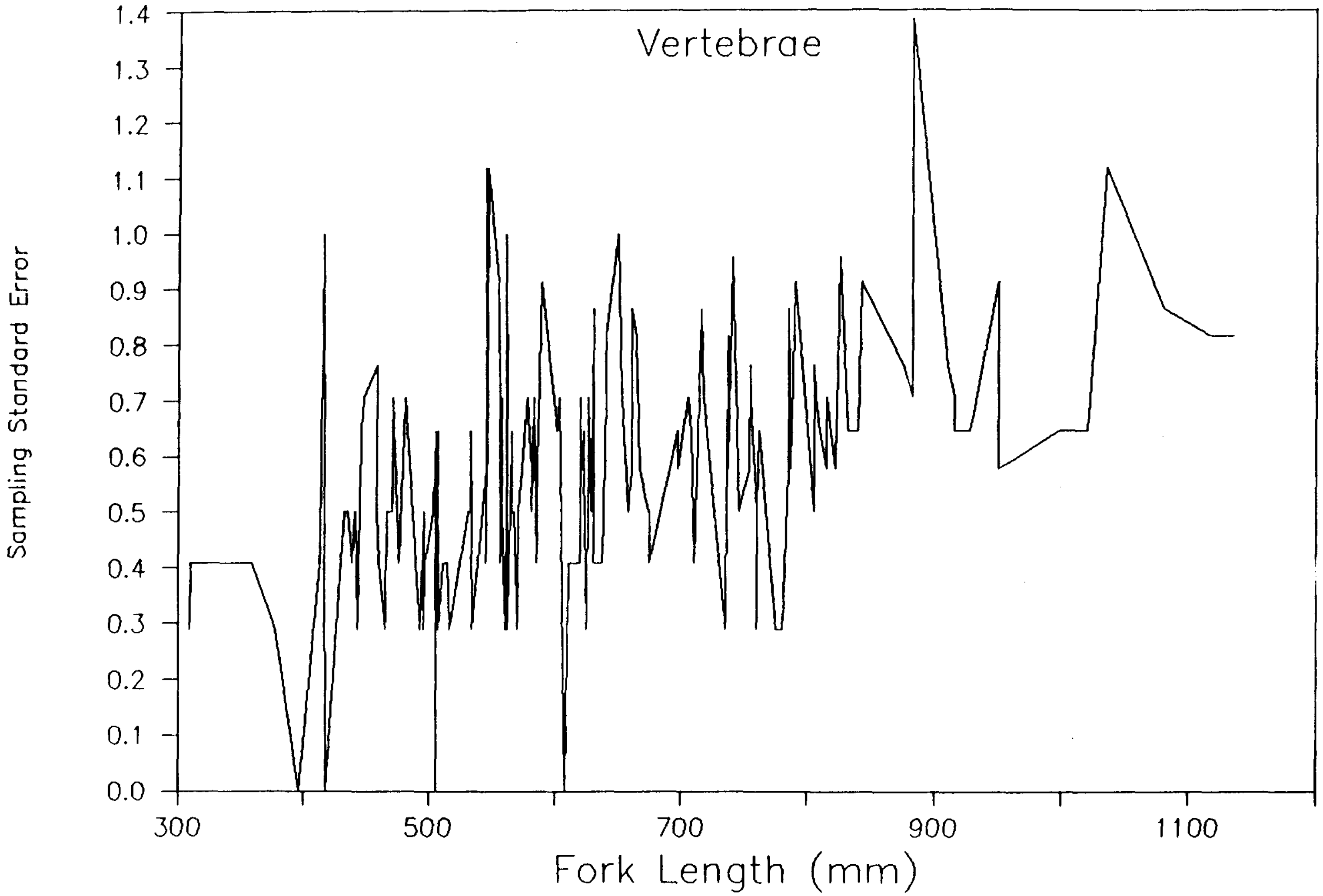

Figure 7. Sampling standard error by the age determinations of 156 burbot based upon annuli counts of vertebrae.

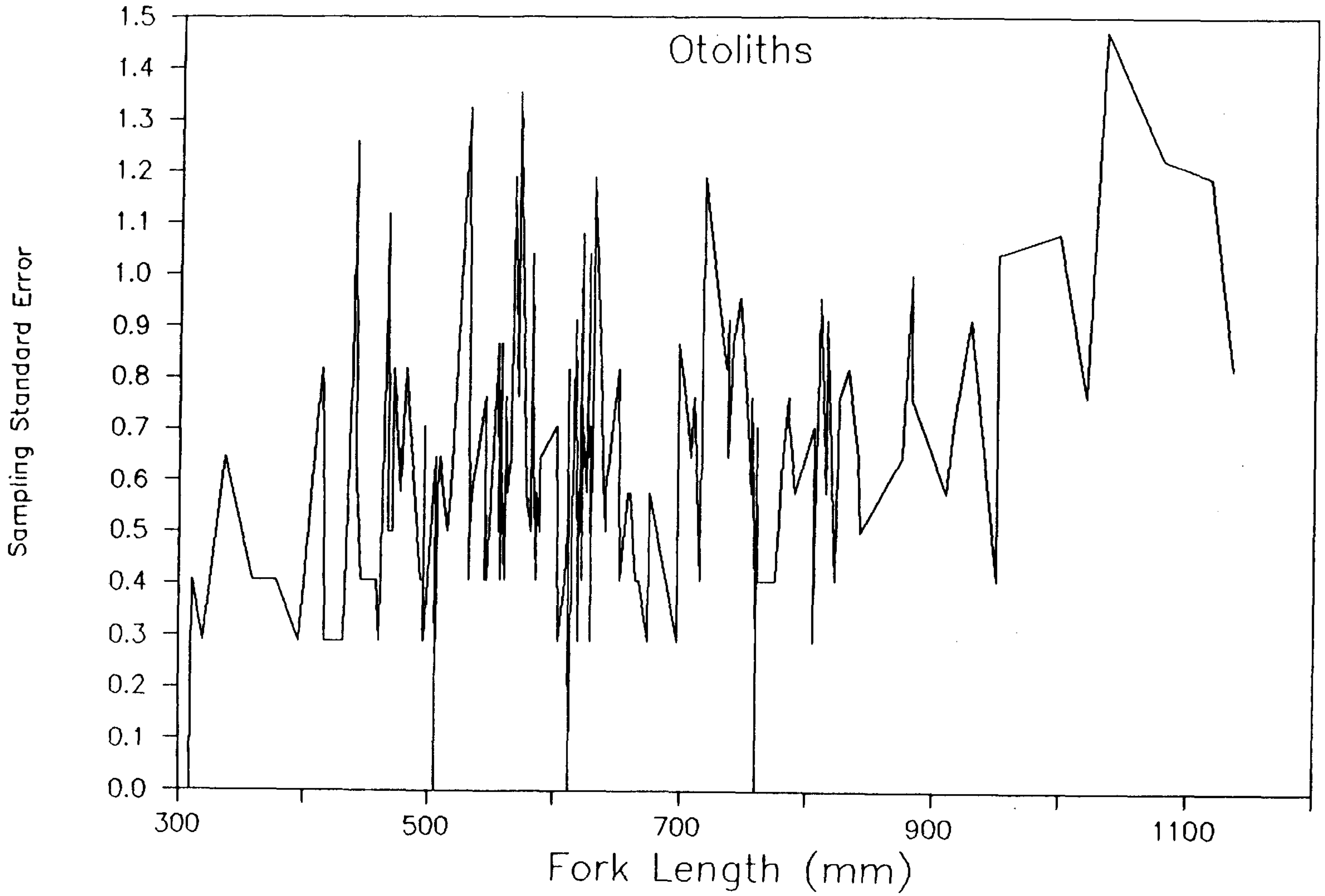

Figure 8. Sampling standard error for the age determinations of 156 burbot based upon annuli counts of otoliths.

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