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# **Evaluations of Various Structures for Use in Age Determination of Arctic Grayling**

by

**Margaret F. Merritt  
and  
Douglas F. Fleming**

October 1991

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Alaska Department of Fish and Game

Division of Sport Fish



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EVALUATIONS OF VARIOUS STRUCTURES  
FOR USE IN AGE DETERMINATION OF  
ARCTIC GRAYLING<sup>1</sup>

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## ABSTRACT

Precision of ages determined from scales, sectioned pelvic fin rays, whole and sectioned vertebrae, whole and sectioned opercula, and whole and broken-and-burned otoliths was measured in three trials among four readers on samples from Arctic grayling *Thymallus arcticus*. A validation study evaluated the ability of two readers to detect annular growth from Arctic grayling scales formed during a one-year hiatus between tag and recapture events. Analysis of variance models were used to determine differences in estimated mean age among structure and reader, and in validating a one-year age difference. Estimated ages from whole otoliths were the most precise, and significantly older than other structures. Estimates of mean ages among structures varied significantly among three of four readers. There was no significant interaction between reading time and precision of ages in otoliths. A large portion of the variation in ages determined from scales was explained by reading time, although significance of this correlation was not great. Reader experience contributed to a lack in precision in the mean age across structures. Significant annular growth was detected in the validation study, although failure to detect the addition of one annulus occurred about 50 percent of the time. The distribution of the error in detecting a one year change in age was equally divided between negative and positive biases, resulting in a net bias of 1.1 percent for the sample.

KEY WORDS: Arctic grayling, *Thymallus arcticus*, age validation, precision, aging, scales, pelvic fin rays, vertebrae, opercula, otoliths, reader experience, reading times.

## INTRODUCTION

Criteria for determining ages of Arctic grayling *Thymallus arcticus* have not been comprehensively evaluated. Evaluating the precision with which annuli can be counted is necessary because imprecise ages may lead to errors in estimates of growth rate and production (Ricker 1975) as well as estimates of mortality (Barlow 1984). Evaluating the ability of a reader to detect an annulus formed between tagging and recapture after a one year interval is one method of testing the reliability of aging (Beamish and McFarlane 1983). A drawback to using mark-recapture studies in validating age is that the detection of annulus formation does not prove that the age is accurate. Only the use of known-age fish will provide accurate ages.

Studies comparing scale and otolith ages have been conducted on Arctic grayling by Craig and Poulin (1975), Schmidt and Stratton (1984), McCart et al. (1972) and deBruyn and McCart (1974). Sikstrom (1983) compared otoliths, pectoral fin rays and scales for age estimates among two readers. All authors found that among older fish, otolith and fin ray ages were generally greater than scale ages. No comparisons of age estimates among scales, otoliths, vertebrae, opercula and pelvic fin rays in Arctic grayling have been reported in the literature. The ability of readers to consistently repeat counts of annuli among Arctic grayling structures has not been examined in the literature, either. The studies cited above have focused only on one measure of variation in counts, yet precision is a "catch-all" term that has many statistical definitions.

The use of mark and recapture studies to validate age estimates has been discussed by several researchers, including Beamish and McFarlane (1983), Alvord (1953), Mills and Beamish (1980), Quinn and Ross (1982), and Matlock et al. (1987). The only known scale validation study for the genus *Thymallus* (*T. montanus*) was by Brown (1943). No study of scale age validation for *T. arcticus* has been reported in the literature.

Arctic grayling from interior Alaska were sampled to evaluate the precision of estimated ages using scales, whole and broken-and-burned otoliths, whole and sectioned vertebrae, whole and sectioned opercula, and sectioned pelvic fin rays. The ability to detect annulus formation in scales using fish resampled in tagging studies was also examined. Objectives within the precision study were to: determine the most precise structure(s) for aging Arctic grayling; examine variation around estimated mean ages by readers and structures; and, examine the effects of reader experience and reading times on variation about the mean age for different structures. The objectives for the validation study were to: determine if an additional annulus can be detected on scales removed from tagged Arctic grayling one year after initial scale samples were obtained; and, examine variation in age detection among readers. Scales were selected for the validation study because they are the most commonly used structure for aging fish and scale collection is not lethal.

## PRECISION STUDY METHODS

### Sample Selection

Two-hundred and seventy mature and immature Arctic grayling were collected by hook and line, beach seine and electrofishing during August and September 1988 from the Chatanika (65° 06'N, 147° 28'W) and Delta (64° 09'N, 145° 51'W) rivers in interior Alaska. A stratified random subsample of 12 fish from the Chatanika River and 13 fish from the Delta River was selected for this study. The 25 fish were evenly distributed among five length classes ranging from 150 mm to 399 mm fork length. The subsample was chosen without regard to fish sex as gender was assumed to have no effect on precision of age estimates. In addition, the sample was collected at a time of year when sex of fish was not discernable.

### Structure Preparation

Two scales per fish were taken approximately six scale rows above the lateral line, just posterior to the anterior insertion of the dorsal fin on the left side of each fish. Scales were cleaned and an acetate impression made which was examined with a microfiche viewer (magnification = 35 x). Annuli on scales (Figure 1A) were identified according to criteria outlined by Yole (1975). Whole sagittal otoliths were cleaned, air-dried, and examined on a dark background with a dissecting microscope (magnification = 12 to 25 x) with diffused lighting. Annuli on whole otoliths (Figure 1E) were identified as calcified ridges and counted from the kernel outward, similar to criteria followed by Sikstrom (1983). Three vertebrae were removed from the vertebral column of each fish at a location anterior to the insertion of the dorsal fin. They were immersed for three minutes in boiling water, then scrubbed clean with a brush. Vertebrae centra were examined like otoliths, except for differences in viewing (magnification = 6 x). Opaque bands on whole vertebrae (Figure 1C) were counted as annuli from the focus of the centrum to the edge. Ages were estimated using criteria reported by Prince et al. (1985). Opercula were cleaned like vertebrae, then immersed for 10 seconds in diluted malachite green ( $\approx$  1:10). Visual contrast between opaque and translucent bands was increased because the opaque bands stained a darker green. Examination of opercula was similar to vertebrae. Age estimates from whole opercula were obtained by counts of each opaque growth ring from the fulcrum diagonally to the edge (Figure 1B), consistent with Le Cren (1947). Three pelvic fin rays were removed from the medial portion of the left pelvic fin and cleaned as were vertebrae. Fin rays were embedded in epoxy and sectioned following methods of Sikstrom (1983). Sections taken from the base of each ray varied between 0.5 and 0.9 mm in thickness. Sections were mounted on glass slides and examined under a compound microscope (magnification = 200 x). Age estimation of sectioned fin rays (Figure 1D) followed the criteria of Beamish (1973) and Sikstrom (1983).

### Experimental Design

Four readers of varying levels of experience in aging fish (Table 1) were instructed on interpretation of annuli in all structures prior to the study so that criteria for aging was standardized. Scales, whole vertebrae, otoliths

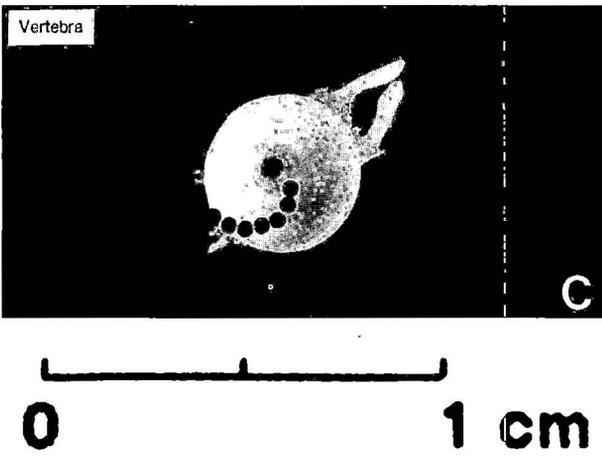
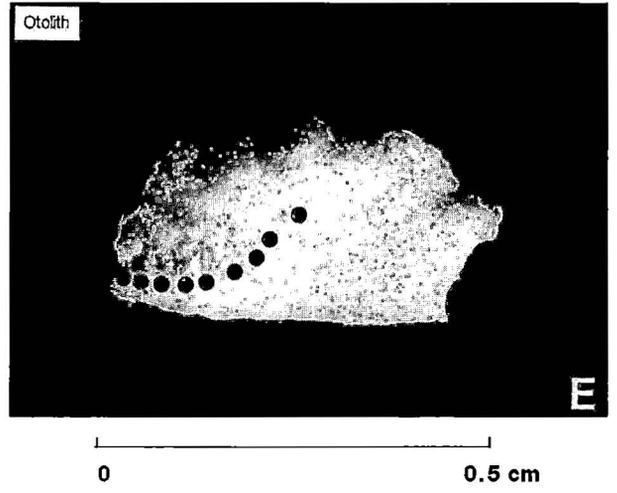
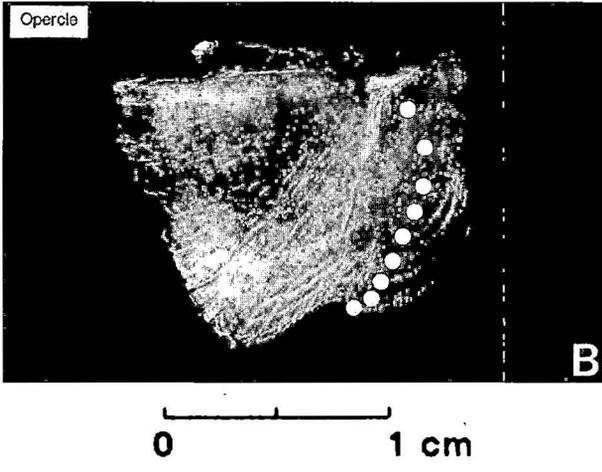
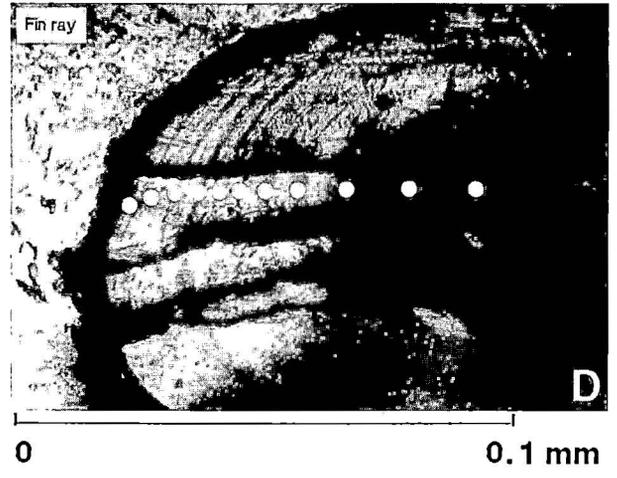
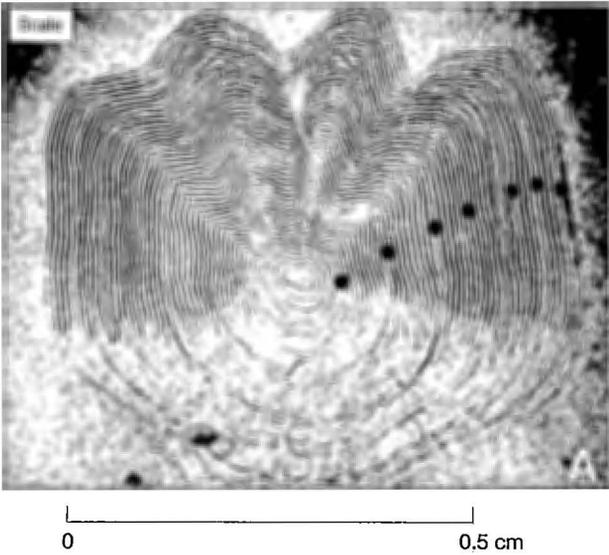


Figure 1. Photographs of five structures used in estimating age from Arctic grayling. Black or white dots indicate annuli.

Table 1. Ratings of reader experience in estimating age by structure, where 0 = no experience, 1 = experience with other species, and 2 = experience with Arctic grayling.

Reader	Scales	Otoliths	Vertebrae	Opercula	Fin Rays	Mean
1	1	0	1	0	0	.2
2	1	1	1	1	1	1.0
3	2	2	1	0	0	1.0
4	2	2	2	1	0	1.4
Mean	1.5	1.3	1.3	0.5	0.3	

and opercula, and sectioned fin rays were aged from 25 Arctic grayling. The same four readers aged broken-and-burned otoliths, and sectioned vertebrae and opercula from the subsample for comparison with whole structure annuli counts. Each structure-fish combination was repeated three times (four readers x eight structures x 25 fish x three replicates = 2,400 estimates). The order of structures examined, and fish within replicates was randomized. Time required to read each replicate of 25 fish was recorded. Readers were not informed of fish lengths in the study, but knew all fish were collected at a similar time.

### Data Analysis

Precision of age estimates has been described in various ways, such as the maximum difference among reader means, sampling standard error, coefficient of variation, average percent error, and percent agreement (Sharp and Bernard 1988, Beamish and Fournier 1981, Chang 1982). Each measure of variation about the mean has advantages and disadvantages relative to what the investigator is attempting to demonstrate.

Following the philosophy expressed in Chang (1982), comparisons of the relative precision of estimates among structure-reader combinations were based on the coefficient of variation (CV). While Chang's CV is based upon variation across replicated age estimates specific to individual fish, it was our desire to observe the CV of each reader-structure combination for descriptive purposes. We propose the following:

$$(1) \quad CV[\bar{x}_{sp}] = \left[ \frac{\sum (\bar{x}_{spf} - \bar{x}_{sp})^2}{(F - 1) \bar{x}_{sp}^2} \right]^{1/2} \times 100$$

where

$$\bar{x}_{spf} = \frac{\sum x_{spf r}}{R}$$

$$\bar{x}_{sp} = \frac{\sum \bar{x}_{spf}}{F}$$

and where  $x_{spf r}$  is the rth estimate from fish f, by person p from structure s. The subscripts s, p, f, and r take on values from one to S, P, F, and R, respectively. The structure with the lowest CV was judged as giving the highest precision.

Comparisons in repeatability of estimates for structure-fish combinations were based on the sampling standard error (SSE). This is because the SSE measures variation in the distribution of reader sample means (Zar 1984). SSE was

calculated after Sharp and Bernard (1988). SSE was used to correlate measurement error with fish length.

Analysis of variance (ANOVA) was used to test the significance of differences in estimates of mean age among structures and readers. The ANOVA was based on a general linear model for a balanced factorial design with structures as fixed effects and readers as random effects. Fish were used as a blocking variable. While the ANOVA is considered to be robust for multisample testing among means, homoscedasticity among structures (an assumption of the ANOVA) was examined with Bartlett's Test (Zar 1984). A finding of unequal variances in estimates of mean age among structures ( $B_c = 53.8$ ,  $df = 19$ ,  $P < 0.001$ ) reduced the power of the ANOVA to detect a difference in age estimates. This reduction in power resulted in the discrimination of differences in mean ages of 0.4 year among structures. The detection of a difference in age of 1.0 year is considered to be sufficient for biological significance. Since sample sizes were equal among variables, the ANOVA was considered robust even with significant heterogeneity (Zar 1984). Residuals were examined, and suggested no violation of general linear model assumptions.

## VALIDATION STUDY METHODS

### Sample Selection

Acetate scale impressions from 94 Arctic grayling tagged in Fielding Lake (63°10' N, 145°58' W) and the Chatanika, Chena (64°50' N, 147°10' W), and Salcha (64°30' N, 146°35' W) rivers of interior Alaska in 1986 and 1987, and recaptured approximately one year later (time "t+1"), were selected for this study. The fish in the sample ranged from 165 mm to 390 mm in fork length, characteristic of Arctic grayling size distributions for interior Alaska. Fish were collected using a pulsed DC electrofishing boat during both sampling events. Arctic grayling greater than 150 mm FL were marked with individually numbered Floy internal anchor tags inserted at the base of the dorsal fin. The tip of a selected fin (either adipose, pectoral, pelvic or caudal) was removed to identify marked fish in case the tag was shed. This method assumes tagging has no effect on growth.

### Experimental Design

Two readers with relatively little aging experience were instructed on interpretation of scale annuli to standardize aging criteria prior to the study. Population source and sample collection dates were available to the readers to assist with interpretation of annuli formation. Numbered acetate impressions of scales were randomly ordered. Ages were estimated for 94 fish, at times t and t+1, by two readers twice each (94 fish x two years x two readers x two replicates = 752 estimates).

### Data Analysis

Analysis of variance (ANOVA) was used to validate the addition of an annulus on scales. A balanced repeated-measures experimental design allowed hypothesis testing at the detection of a one-year age difference. The model held fish,

readers, and year as random effects, and trial was used as the error term. The model was:

$$\text{Age} = \mu + \text{Fish} + \text{Reader} + \text{Fish*Reader} + \text{Trial}(\text{Fish*Reader}) + \text{Year} + \text{Fish*Year} + \text{Reader*Year} + \text{Reader*Fish*Year} + \epsilon$$

Post ANOVA analysis included Duncan's Multiple Range Test to compare means of age estimates among readers and years. The mean detected age difference between times t and t+1 year was calculated for all reader-fish combinations. Growth increments between sampling events were tabulated and correlated against reader mean age differences to examine age increment failures.

The proportion of fish aged as one year older in time t+1 was calculated as follows. Each replicate reading was treated as an independent observation, so that for a given fish, there were four estimates of age at both times t and t + 1. Because replication is not part of our normal aging procedures where fish are generally aged once, the data was reduced randomly to one observation per reader per time. The percent validation was calculated as the frequency at which a fish was aged one year older.

## RESULTS

### Precision and Repeatability

The CVs among structure-reader combinations (Table 2) ranged from 26% (reader 1; whole opercula) to 56% (reader 4; sectioned vertebrae). The smallest mean CVs among readers was observed in whole and broken-and-burned otoliths (33%). Repeatability in age estimates (as measured by the SSE) appeared independent of fish length for all structures examined except broken-and-burned otoliths (Figure 2). Readers made more "mistakes" in repeating annuli counts for broken-and-burned otoliths as fish length increased. Because of this, they were removed from further consideration in the study.

### Estimates of Mean Age

No significant difference ( $P = 0.25$ ) was found between estimates of mean age from whole and sectioned opercula. Since processing of whole opercula did not improve the CVs (Table 2), increased laboratory time for processing was the deciding factor in choosing to remove sectioned opercula from further consideration in the study. A significant difference in mean age estimates ( $P < 0.008$ ) was found between whole and sectioned vertebrae. Sectioning tended to obscure the outer, closely-spaced annuli which resulted in a shift in count distribution towards younger ages (Figure 3). For this reason, sectioned vertebrae were removed from further study, leaving five structures for a reduced analysis: scales, sectioned fin rays, and whole otoliths, opercula and vertebrae.

Estimates of mean age showed significant differences ( $P < 0.0001$ ) among five structures and four readers (Table 3) in the reduced ANOVA model. A multiple comparison test based on least significant differences grouped otoliths apart from other structures. The estimated mean age from otoliths (6.4 years) was

Table 2. Mean counts of annuli, coefficient of variation (%), and sampling standard error for each reader-structure combination.<sup>a</sup>

Structure	Reader				Mean
	1	2	3	4	
<b>Scales</b>					
Mean	5.8	4.7	5.0	5.4	5.2
CV	33	35	43	44	39
<b>Whole Otoliths</b>					
Mean	6.9	6.0	5.9	6.8	6.4
CV	30	33	29	36	33
<b>Broken Otoliths<sup>b</sup></b>					
Mean	6.6	6.0	5.4	5.9	6.0
CV	28	31	35	38	33
<b>Whole Vertebrae</b>					
Mean	6.2	5.7	5.6	6.3	6.0
CV	40	46	43	46	44
<b>Sectioned Vertebrae<sup>b</sup></b>					
Mean	5.4	5.0	5.2	5.5	5.3
CV	43	39	48	56	47
<b>Whole Opercula</b>					
Mean	6.1	5.4	4.8	5.8	5.5
CV	26	34	36	43	35
<b>Sectioned Opercula<sup>b</sup></b>					
Mean	6.4	5.3	5.1	5.8	5.6
CV	31	32	39	42	36
<b>Fin Rays</b>					
Mean	6.5	5.2	5.4	5.8	5.7
CV	36	39	43	46	41

<sup>a</sup> Each mean is calculated from 75 observations (25 Arctic grayling read 3 times).

<sup>b</sup> Dropped from further study.

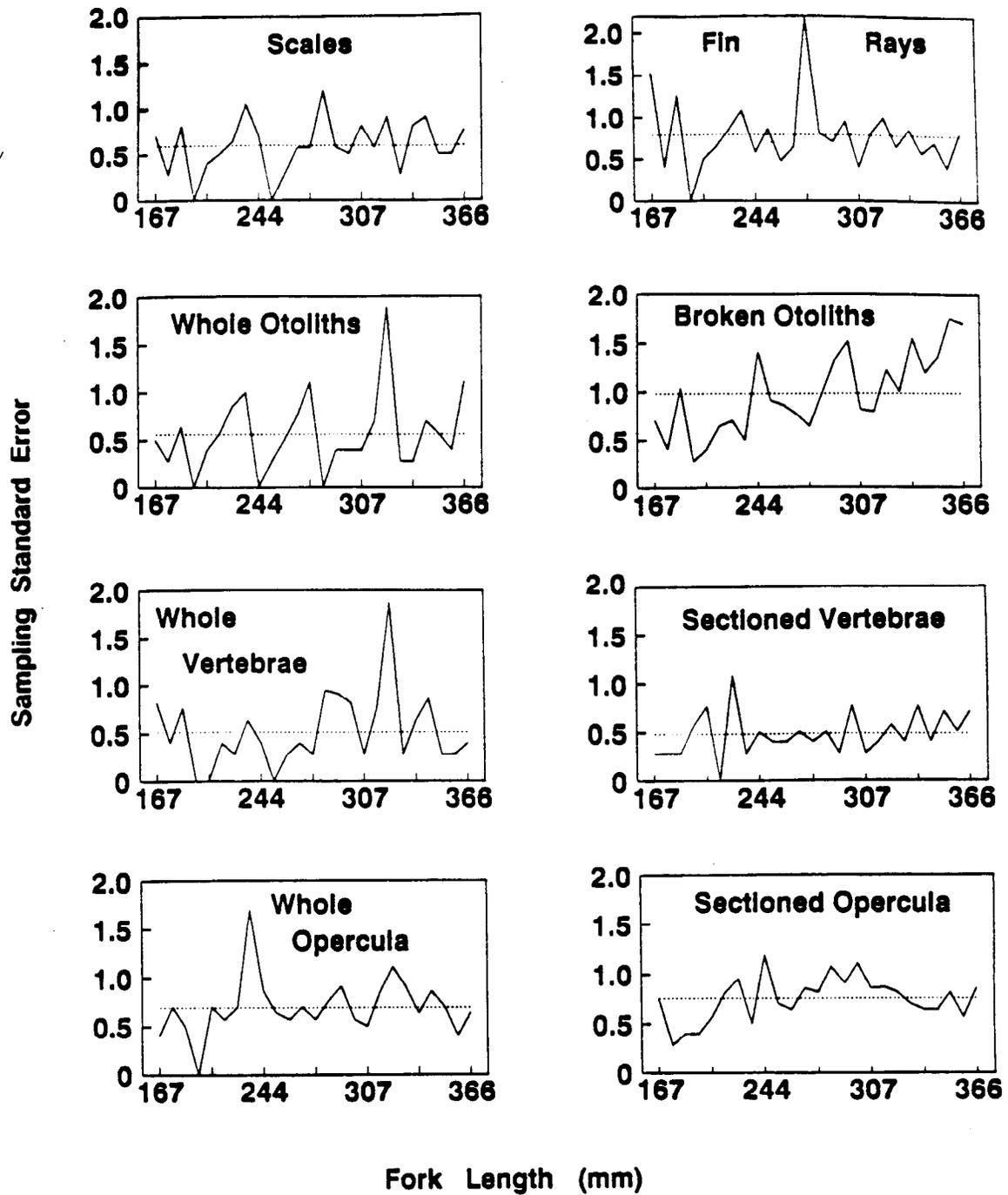


Figure 2. Sampling standard error by fork length for all structures. The dashed line represents the mean sampling standard error for that structure.

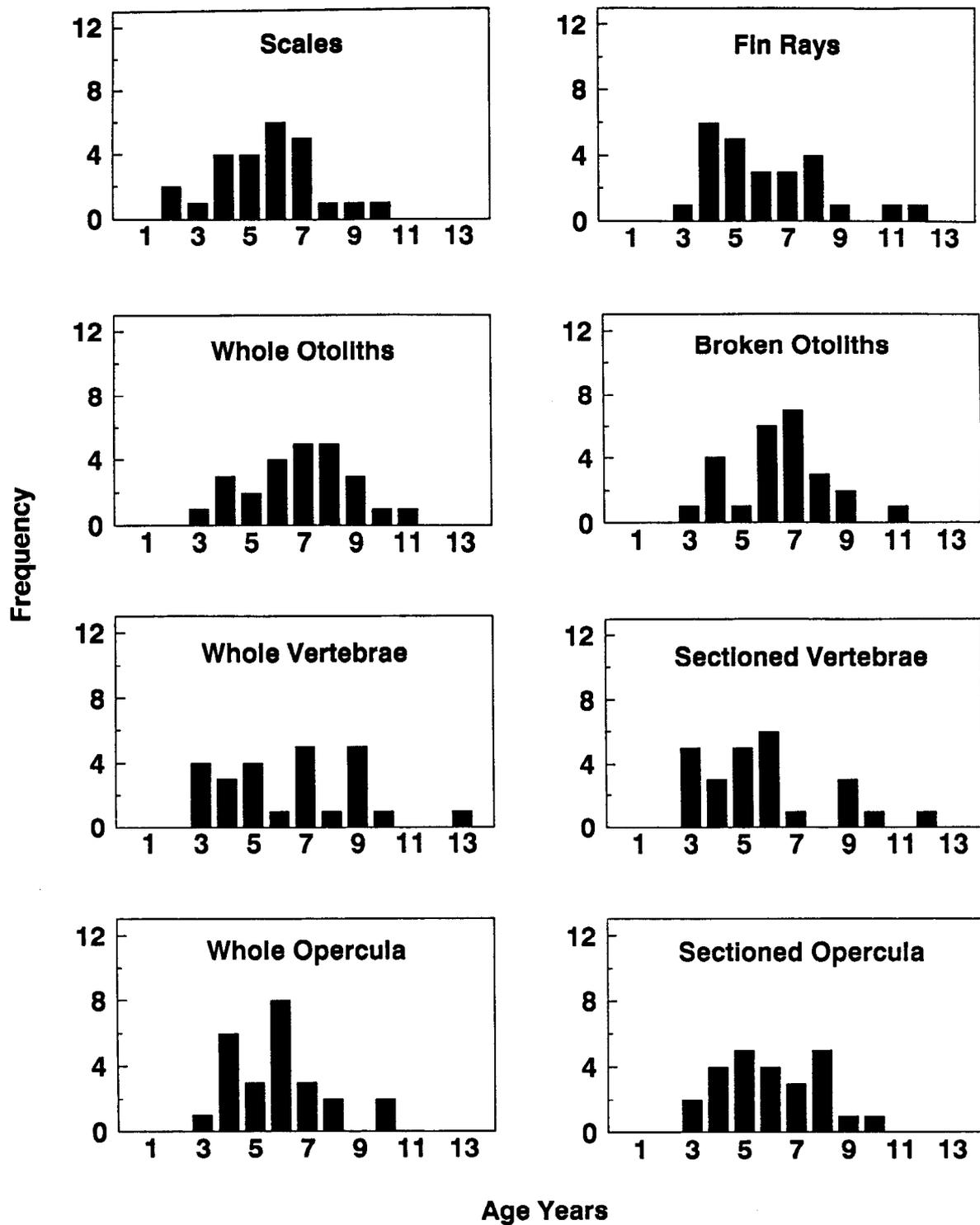


Figure 3. Mean age frequency distribution of the 25 Arctic grayling studied, by structure examined. (Each fish was aged 12 times, and the histogram represents the frequency of occurrence of the means).

Table 3. Analysis of variance comparison of age estimates among readers and structures<sup>a</sup>.

Source	DF	SS	F	Probability
Fish (F)	24	5,944.8		
Reader (R)	3	251.6	27.1	<0.00001
Structure (S)	4	235.7	26.8	<0.00001
RS	12	37.2	1.4	0.16213
RSF	455	1,016.3		
Sampling Error	990	563.2		

Reader	1	4	2	3
Mean	6.3	6.0	5.4	5.4

Structure	Otolith	Vertebra	Fin Ray	Opercle	Scale
Mean	6.4	6.0	5.7	5.5	5.2

<sup>a</sup> Groups of means connected by a line represent insignificant differences in age estimates among readers or structures.

significantly older than those from all other structures, differing by as little as 0.4 year with vertebrae and as much as 1.2 years with scales.

A multiple comparison test indicated that the four readers were divided into three groups (Table 3). Estimates of mean age of all structures by reader ranged from 5.4 to 6.3 years. Power of the ANOVA discerned differences as small as 0.3 years among readers, with the largest mean difference at about one year.

#### Reader Experience and Reading Times

Reader experience ranged from familiarity with reading two of the five structures for other species to expertise in reading three of the five structures for Arctic grayling (Table 1). Scales were the structure with which readers had the most experience. While a correlation of mean age estimates among readers and experience was not conducted, there appeared to be no relation between the two. The two oldest estimates of mean age (6.3 and 6.0 years) were from the least and most experienced readers, respectively.

Readers became more efficient as they gained experience in reading structures. The first, second and third replicates (readers combined) required a total of 27.9, 21.9, and 18.9 hours, respectively, for all structures. The third replicate reading time was considered representative for comparison of reading times among structures. Reading times significantly differed among structures (Friedman Rank Sum Test;  $S = 8.15$ ,  $0.02 < P < 0.05$ ). Whole vertebrae, scales and whole opercula required the least amount of time to read. Sums of reader times combined per structure were 2.1, 2.2 and 2.4 hours, respectively. Whole otoliths and sectioned fin rays required the most time to read (3.0 hours each).

Relations between reader experience, reading times, and relative precision (CV) were examined with a spearman rank correlation. There was not a significant correlation between reader experience and reading times ( $r = -0.42$ ,  $P = 0.068$ ). There was not a strong correlation between reader experience and precision within structures ( $r = 0.12$ ,  $P = 0.065$ ), however the sum of experience across structures showed a significant positive linear relationship with the sum of each reader's CV about the mean age ( $r = 0.99$ ,  $P < 0.001$ ).

Precision was positively correlated to reading times for sectioned fin rays ( $r = 0.99$ ,  $P = 0.003$ ). There were not significant correlations between precision and reading times for opercula ( $r = 0.85$ ,  $P = 0.076$ ) and scales ( $r = 0.78$ ,  $P = 0.110$ ). Precision of ages determined from sagittal otoliths and vertebrae was insensitive to variation in reading times ( $r = 0.13$ ,  $P = 0.433$ ;  $r = 0.05$ ,  $P = 0.475$ , respectively).

#### Validation of Scale Annuli

The estimated mean age across readers was 5.7 years at time  $t$  and 6.7 years at time  $t+1$ . The difference in mean age of one year was significant (ANOVA  $P = 0.0001$ , Table 4).

Table 4. Repeated measures analysis of variance for comparison of mean ages estimated by year and reader.

Source	DF	SS	F	Probability > F
Fish	93	1,947.3		
Reader	1	5.1	5.6	0.0190*
Reader*Fish	93	113.4	1.11	0.2727*
Trial (Reader*Fish)	188	174.5	-	
Year	1	208.5	406.2	0.0001
Fish*Year	93	102.9	2.1	0.0001
Reader*Year	1	0.3	0.6	0.4165
Reader*Fish*Year	93	60.6	1.3	0.0852
Error	188	96.5		
Reader	1	2		
Mean	6.14	6.30		
Year	1 ("T")	2 ("T+1 Year")		
Mean	5.69	6.74		

<sup>a</sup> Means not connected by a line represent significant differences in age estimates among readers or years.

\* Tests of Hypothesis using the Anova MS of Trial(Reader\*Fish) as an error term.

The change in estimated mean age of a fish at times  $t$  and  $t+1$  should have approximated one year. The percentage of scales for which one additional annulus was detected at time  $t+1$  was calculated from observations assumed to be independent - this is termed the percent validation. For reader 1, percent validation in replicates 1 and 2 was 28% and 50%, respectively. Percent validation for reader 2 in replicates 1 and 2 was 50% and 52%, respectively. The percent validation achieved through random subsampling, to simulate a single reading by each reader, was 44% (Figure 4). The net aging error (the difference between summed positive and negative change-in-age frequencies) was quite small (1.1%).

## DISCUSSION

### Precision, Repeatability and Estimates of Mean Age

Consideration of a structure which offers consistency in estimates of age is important because readers can vary between and within years for a given population study. Readers were most precise in annuli counts for whole otoliths. The lack of interaction between the precision of age estimates from otoliths and reader experience or reading time in the ANOVA model indicates the reliability of otoliths as an aging structure.

It was surprising to find that variation (SSE) in estimates of age using broken-and-burned otoliths increased with fish length. In addition to this source of error, sagittal otoliths from Arctic grayling were small (2 to 6 mm) and fragmented easily. The break-and-burn method was not considered for future use because of increasing SSEs with fish length, fragmentation, and additional lab processing time. Some studies have reported superior results with sectioned or broken-and-burned otoliths in other species. Samuel et al. (1987) reported improved clarity in reading annuli for Indian flathead *Platycephalus indicus* using broken-and-burned otoliths. Beamish (1978) recommended sectioning of otoliths for estimating ages in Pacific hake *Merluccius productus* and Beamish and McFarlane (1987) suggested sectioning otoliths for older sablefish *Anoplopoma fimbria* and lake whitefish *Coregonus clupeaformis* because of increased thickening of the otolith with age.

Williams and Bedford (1973) point out that wide variations in the formation of alternate opaque and hyaline zones can occur in otoliths from year to year. This variation may be due to climatic conditions, geographic location and fish age. These factors should be taken into consideration when using otoliths for aging studies. In this study, otoliths were collected from a wide range of age groups (approximately 2 to 13 years) and two stocks. While it is a possibility that zone variations among ages and stocks may have confused readers and contributed to imprecision in mean age estimates, imprecision was modest compared to that found in other structures examined in this study.

Consistent with other studies, we found ages estimated from whole otoliths were significantly older than from other structures examined. Estimated mean ages based upon otoliths were the oldest in studies comparing otoliths to scales in Arctic grayling (Craig and Poulin 1975, Schmidt and Stratton 1984), and to scales and fin rays in Arctic grayling (Sikstrom 1983). Sikstrom

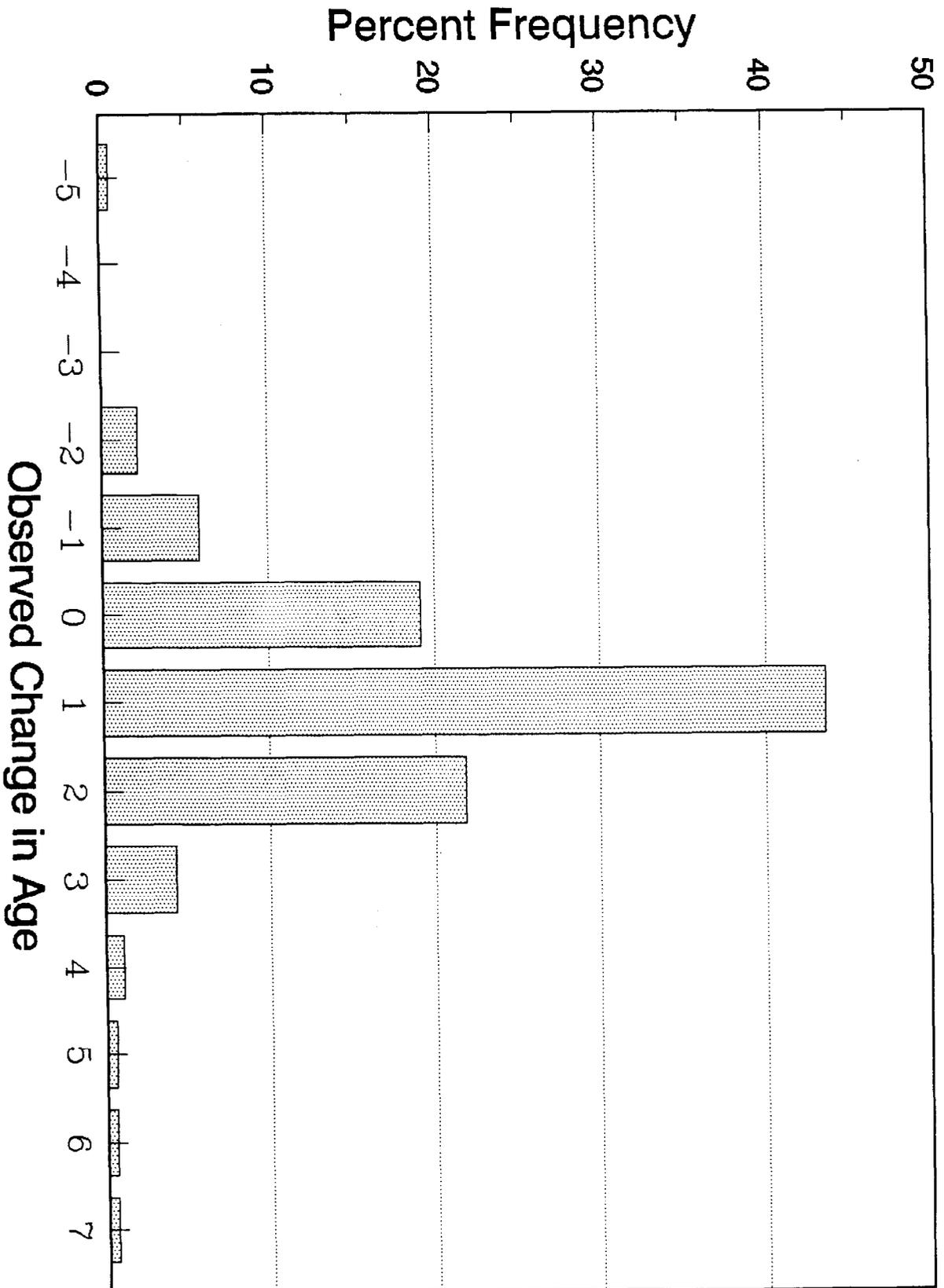


Figure 4. Observed change-in-age from time at tagging (t) to recapture (time t+1) for 94 Arctic grayling by two readers (n = 188).

theorized that the disagreement between age estimates from otoliths and fin rays was due to difficulty in locating the first fin ray annulus, and related this to problems encountered in a study of slow growing white suckers *Catostomus commersoni* by Beamish (1973). The findings of both researchers point out that significantly older ages in a given structure may be the result of processing or interpretation of annuli in other structures.

Another factor which may have contributed to older age estimates from otoliths is splits in hyaline rings termed "false" rings. The presence of splits is reported by Williams and Bedford (1973) to be the main source of difficulty in the interpretation of otolith age. Until it is discovered why whole otoliths provided a significantly greater age than other structures examined in this study, it will remain in doubt as to whether greater annuli counts represent more years. Validation of Arctic grayling age estimates using otoliths is recommended.

Differences in estimates of mean age among readers was greater for pelvic fin rays and whole opercula than for scales. This finding varies from that of Sikstrom (1983) who found percent agreement among readers to be greater for pectoral fin rays in Arctic grayling than for scales. Differences in the findings of these studies might be related to structure (pelvic versus pectoral fin rays), sectioning technique, or experimental design. This study found fin ray ages to be significantly older than scales, and significantly younger than whole otoliths. These results are consistent with Sikstrom (1983) in Arctic grayling (scales and otoliths) and with Mills and Beamish (1980) in lake whitefish (scales only). Fin rays can be removed from a fish without killing it and for this reason fin rays offer an advantage over the collection of otoliths, opercula and vertebrae. However, based on the results from this study, fin ray collection for aging Arctic grayling is not recommended because of high CVs and large disagreements among readers in mean counts.

Scales are the most common structure used to age fish, although it has been recently shown that scales often tend to underestimate ages (Beamish and McFarlane 1987, Craig and Poulin 1975). In this study, scales were aged significantly younger than all other structures except opercula. The advantage to using scales for age estimates is that scale collection is not lethal. The disadvantage to using scales for aging is that estimates of stock production may be exaggerated.

#### Reader Experience and Time

All readers were briefed regarding interpretation of annuli, so it was surprising that estimates of mean age significantly differed among three of four readers. The range of mean age estimates among readers was not a reflection of experience. Obviously, the instruction on interpretation of annuli given to readers prior to the study did not standardize criteria for pattern recognition in the structures examined. Since personnel who determine fish ages from scales can vary from year-to-year, discrepancy in discerning annuli among readers could affect stock assessment estimates. Carlander (1987) outlined the difficulties in recognition of scale annuli and stated that results of age class compositions in the early years of fisheries science

were just best guesses. The results of this study suggest that the difficulties in recognition of annuli have not been ameliorated for Arctic grayling.

We hypothesized that readers, experience in aging fish, and the amount of time spent aging were equal variables and used readers in the ANOVA to determine differences in estimated mean age by structure. Close examination of these variables revealed, for the most part, minimal correlations. About 10% of the variation in reading times and precision among structures was explained by reader experience. In regards to the effects of reading times on precision among structures, virtually no correlations were detected for sagittal otoliths and vertebrae. Since otoliths were the most precise structure in determining ages, it is reassuring that the measure of precision was not confounded. A large portion of the variation in ages determined from scales (61%) and opercula (73%) was explained by reading times, however the significance of these correlations is not great ( $P > 0.05$ ). Significantly less variation in aging fin rays occurred with increasing reading time, suggesting that a learning curve for pattern recognition in fin rays was developing in readers. Since fin rays are not recommended for use in aging Arctic grayling, this confounding interaction is not of much import.

One measure of precision (the sum of each reader's CV about the mean age across structures) was inversely correlated with the sum of experience across structures. This suggests that a person's total experience in aging fish contributes to a lack of precision. It may be that in this study, those readers who were most experienced were overly confident, and did not ensure consistent application of criteria in recognition of annuli.

#### Validation of Scales

Using a mark-recapture approach, we were able to detect the formation of a single scale annulus from Arctic grayling after one year of growth. An ANOVA model detected the one year difference in mean age despite the underlying imprecision of the reader, and scales as an aging structure. Although failure to detect the addition of one annulus occurred about 50% of the time, the distribution of the error in detecting a one year change in age was equally divided between negative and positive bias.

#### Conclusions

Scales have been the method of choice for aging Arctic grayling because population studies in Alaska require nonlethal means of structure collection. Also, fishery managers are aware of public support favoring fish survival in stock assessment research. This study points out that the desire to retain scales as a method for aging Arctic grayling is at odds to some extent with the desire to precisely and accurately age Arctic grayling.

Scales provide less precise estimates of age than whole otoliths, however there is only a slight difference in the CVs between the two structures, and a slightly greater margin of error may be an acceptable price to pay considering that scales are a nonlethal means of aging Arctic grayling. There are high levels of imprecision in detecting annulus formation in scales of Arctic

grayling, however the distribution of the error is balanced between underaging and overaging. Because scales provide significantly younger age estimates than other structures, estimates of growth rate and stock production may be exaggerated. The overall experience of readers in aging fish structures influences the precision of aging, however time spent aging is not considered a significant source of variation in scale ages.

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