

## Using Otoliths and Scales to Describe Age and Growth of Yellowstone Cutthroat Trout in a High-Elevation Stream System, Wyoming

### Abstract

Estimates of age and lengths at specific ages of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri* Richardson) were made using otoliths and scales. Fish were sampled from 17 high-elevation streams in the Greybull River drainage, Wyoming. Variation in estimates of age within and among three readers were assessed using both structures. Variability among age estimates by individual readers was low for both structures. Estimates using otoliths were significantly less variable than were estimates based on scales both among readers and among estimates by individual readers. Otoliths were more accurate than scales for estimating the correct age of fish. Back-calculated estimates of fish lengths at given ages based on otoliths were significantly less than those based on scales. Hatchery fish grew faster than wild fish at younger ages. Overall, growth of wild fish was slower than in other areas where Yellowstone cutthroat trout are endemic. We predict that if otoliths were used instead of scales to assess age and growth of other trout species in high-elevation streams that similar differences in estimates based on the two structures would be observed.

### Introduction

As populations of cutthroat trout (*Oncorhynchus clarki* Richardson) continue to decline (Young 1995), accurate estimates of population statistics, including age and lengths of fish at given ages, become more important to managers attempting to maintain and preserve remaining populations (Summerfelt 1987). Changes in age structure or growth rates can be used to detect environmental changes or ecological conditions influencing growth (Hammers and Miranda 1991). Slow growth may indicate limited food resources, overpopulation, competition with another species, or unfavorable environmental conditions.

Historically, ages of cutthroat trout have been estimated from scale measurements (Brown and Bailey 1952; Laasko and Cope 1956; Averett and MacPhee 1971; Hubert et al. 1987). Recently otoliths have been used (Armstrong 1971; Moring et al. 1981; Lentsch and Griffith 1987; Hubert et al. 1987). However, inconsistencies in age estimates based on these structures have been reported (Hubert et al. 1987; Murray 1994; Campana et al. 1995).

Among cutthroat trout, otoliths reportedly provide better estimates of age than do scales (Hubert et al. 1987), but little research substantiates this claim. In high-elevation systems, scales are thought to yield inaccurate estimates of age because annuli may not form the first year due to late spawning and the short growing season (Brown and Bailey 1952; Alvord 1954; Bulkley 1961; Averett and MacPhee 1971). Also, in trout older than 3-5 years, scales become difficult to interpret because annuli are in close proximity to each other, and scale erosion and re-absorption may obliterate annuli (Alvord 1954).

Yellowstone cutthroat trout (YSC; *O. c. bouvieri*) occupied the largest geographic range among the non-anadromous subspecies of cutthroat trout (Varley and Gresswell 1988), but drastic reductions in their distribution have generated concern about the preservation of genetically pure YSC. Hybridization with and displacement by exotic salmonids and habitat destruction (Gresswell 1988) are estimated to have reduced the range of YSC by 90% (Varley and Gresswell 1988) leading to designation of YSC as a "species of special concern—Class A" by the American Fisheries Society and a "sensitive species" by the U.S. Forest Service in the Northern and Rocky Mountain regions. Paramount to planning and initiating management is the need to understand the

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ecology of the remaining populations of YSC. Yellowstone cutthroat trout occur in high-elevation streams (Gresswell 1988; Young 1995) and little information exists on age structure, growth, or survival of these populations. Previous estimates of age and growth of YSC have been obtained using scales (Remmick 1981; Kent 1984; Gulley and Hubert 1985), but the accuracy and precision of these estimates are questionable (Hubert et al. 1987; Murray 1994).

Using otoliths and scales, we estimated age and lengths at specific ages of YSC in 17 high-elevation (2300–3050 m) streams in the Greybull River drainage in Wyoming. Our objectives were to: (1) ascertain the precision of age estimates using scales and otoliths, both among readers and among estimates by individual readers; (2) ascertain whether otoliths and scales yield different estimates of age or lengths at specific ages; (3) describe growth of cutthroat trout in one high-elevation stream system based on features of otoliths and scales; and (4) ascertain whether there was differences in growth between introduced hatchery YSC and wild YSC in the drainage.

## Study Area

The Greybull River drains over 2900 km<sup>2</sup> of the eastern Absaroka Mountain Range in northwestern Wyoming. The study area included that portion of the Greybull River drainage within the Shoshone National Forest (Figure 1). Fifty-six perennial tributaries (355 km of total stream length) occur in the 650 km<sup>2</sup> headwater drainage, and 23 of the perennial streams contained YSC in 1994 (Kruse 1995). The Wyoming Game and Fish Department stocked YSC fry in five of the 23 streams (Anderson, Eleanor, Cow, and Venus creek and the upper Greybull River) in 1988. Stocking occurred above barriers (geologic structures at least 1.5 m high) to upstream migration of fish in streams where no wild YSC were known to occur, except in Venus Creek where hatchery YSC were not isolated from wild YSC by a barrier.

The Greybull River and its tributaries are torrential, high-elevation mountain streams with high channel slopes, unstable substrates, and large fluctuations in discharge from spring to late summer. Elevations of streams in the study range from 2300 to 3050 m above mean sea level. Stream gradients are high, ranging from 0.5 to 25% with a mean of 8.5%. Melting snow dominates the annual

hydrograph and results in extremely high spring flows

## Methods

Using back-pack electrofishers, YSC were collected from 17 of the 23 streams which contained cutthroat trout (see Figure 1). Two hundred and fifty-nine YSC were weighed (g) and total length was measured (mm). Sagittal otoliths were extracted (Schneidervin and Hubert 1986) from all these fish. From 100 fish, scales were removed from above the lateral line below the insertion of the dorsal fin (Jearld 1983). Otoliths were mounted with epoxy, distal surface up, on a microscope slide (Mackay et al. 1990) and polished with 600-grit sandpaper to clarify annuli. Scales were wet mounted between a coverslip and microscope slide.

Estimates of age based on otoliths and scales from the same 100 fish were made by three independent readers on three separate occasions to determine among- and within-reader variability (Kimura and Lyons 1991; Campana et al. 1995). On each occasion the structures were evaluated in random order. Upon establishing that otoliths were the more reliable aging structure, otoliths from 159 additional YSC were examined three times by all three readers to enhance the array of samples from the Greybull River drainage.

To estimate age of individual YSC, otoliths and scales were viewed on a video screen using the Wyoming Game and Fish Department's Optical Pattern Recognition System (OPRS; Biosonics 1985). Annuli were identified following criteria of Jearld (1983) and Mackay et al (1990). Once each structure had been independently evaluated three times by all three readers, the readers collaborated and agreed on an age. The nucleus, measurement axis, and annuli were shown on the video screen, and the OPRS measured and recorded the radius and distance from the nucleus to annuli for back-calculation of total length at specific ages. Back-calculation is based on the assumption that growth of the aging structure and increase in total length of the fish are directly proportional (Jearld 1983).

Coefficients of variation (CV; Chang 1982; Campana et al. 1995) were calculated to determine within and among reader variation in age determinations. Coefficients of variation for each reader were based on three separate age estimates assigned to a given fish by the reader. Coefficients

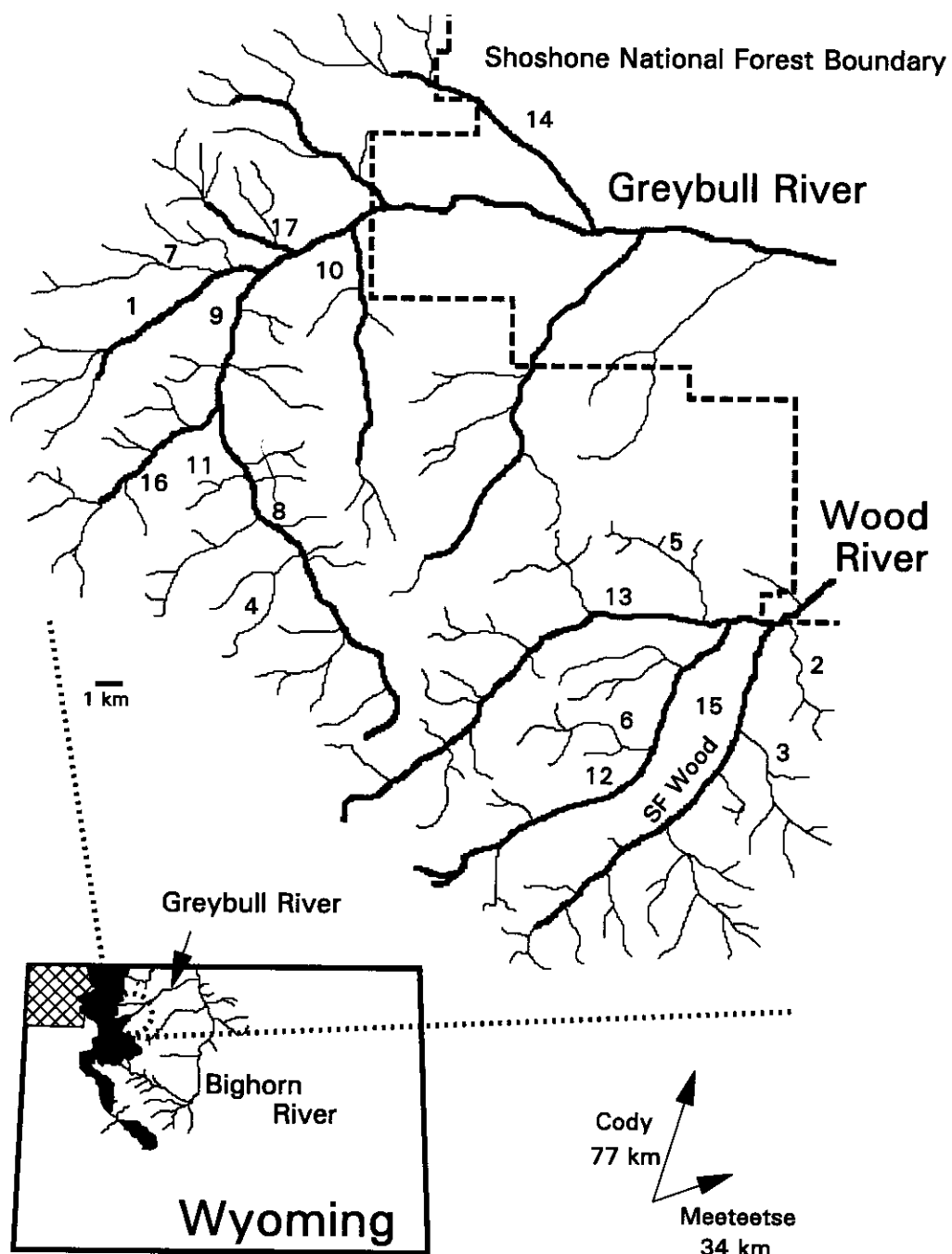


Figure 1. Map showing the location of the Greybull River drainage in Wyoming and location of sampling sites within the drainage. See Table 2 for names of sampling sites corresponding to numbers on the map.

of variation among readers were computed using the mean age (from three readings) assigned to each fish by each reader. One-way analysis of variance (ANOVA; Krebs 1989) was used to detect differences in mean precision (CV) among readers using the same structure and among readings by individual readers using the same structure. Tukey's multiple-comparison test was applied to determine which readers had significantly different CV's.

Age-bias plots were developed to visually assess differences in estimates between readers (Campana et al. 1995). Mean ages determined by one reader were plotted against mean ages determined by a second reader. Pearson correlation coefficients and linear regressions (Neter et al. 1989) between mean ages assigned to otoliths and scales were calculated for each reader.

Mean total lengths at age was used to describe absolute growth of YSC. The direct proportion method was used to back-calculate total lengths (mm) from otolith sections (Lea 1910 in Schramm et al. 1992). Back-calculated lengths from scales were computed using the intercept method (Carlander 1982). A correction factor of 42 mm (length at squamation; Brown and Bailey 1952) was used. Two-sample t-tests (Krebs 1989) were used to test for differences in mean lengths at specific ages, based on otoliths, between samples from hatchery-derived and wild populations of YSC.

Validation of age estimates using otoliths and scales was based on a year class of YSC fry stocked in 1988 above barriers to upstream migration of fish in Anderson, Cow, and Eleanor creeks, and the upper Greybull River. In the summer of 1994, this year class was age six. Since there was no evidence of natural reproduction in these streams, the stocked year class provided known-age fish for validation of ageing techniques.

All statistical analyses were performed using SPSS (SPSS 1991). Significance was determined at  $P < 0.05$  for all tests.

## Results

Coefficients of variation (mean CV) for estimates of age based on otoliths were significantly less than estimates based on scales for all three independent readers (Table 1). No significant differences in mean CV occurred among readers using

TABLE 1. Mean coefficients of variation within and among three independent readers for scales and otoliths.

Reader	Reader precision		
	Scales (n=100)	Otoliths (n=100)	P
	Mean CV	Mean CV	
Within Reader			
1	13.2 <sup>a</sup>	9.55 <sup>b</sup>	0.017
2	11.9 <sup>a</sup>	5.62 <sup>c</sup>	<0.005
3	16.8 <sup>a</sup>	8.80 <sup>b</sup>	<0.005
Between Reader			
Among all 3	17.3	8.40	<0.005
1 vs 2	15.1 <sup>a</sup>	8.52 <sup>b</sup>	<0.005
1 vs 3	21.3 <sup>a</sup>	8.52 <sup>b</sup>	<0.005
2 vs 3	19.3 <sup>b</sup>	9.33 <sup>b</sup>	<0.005

<sup>a,b,c</sup> Mean coefficients of variation with the same alphabetical superscript in each category were not significantly different (Tukey's,  $P < 0.05$ ).

scales. Reader 2 had a lower mean CV than readers 1 and 3 when using otoliths.

The mean CV among the three readers was significantly lower for otoliths than for scales (Table 1). Differences in mean CV between pairs of readers were also significantly lower for otoliths than for scales in all comparisons. No significant differences were found between pairs of readers using the same structure.

Age bias plots revealed differences in age estimates based on otoliths compared to scales, and plots were similar for all readers. For example, the plot of reader 1 versus reader 3 using mean otolith age estimates revealed little difference in ages assigned to otoliths between the two readers (Figure 2, top panel). The regression line did not differ significantly from the 1:1 age ratio reference line indicating little between reader bias. The age-bias plot of reader 1 versus reader 3 for scales revealed differences between the two readers' estimates (Figure 2, middle panel). Reader 3 consistently judged younger fish to be older and older fish to be younger than did reader 1. The bias plot between mean ages assigned to otoliths and to scales by reader 3 revealed that estimates of age using otoliths were consistently higher than those using scales (Figure 2, bottom panel). Readers 1, 2, and 3 had correlations between ages for otoliths and scales of 0.65, 0.67, and 0.46 ( $P < 0.0005$  for all cases), respectively.

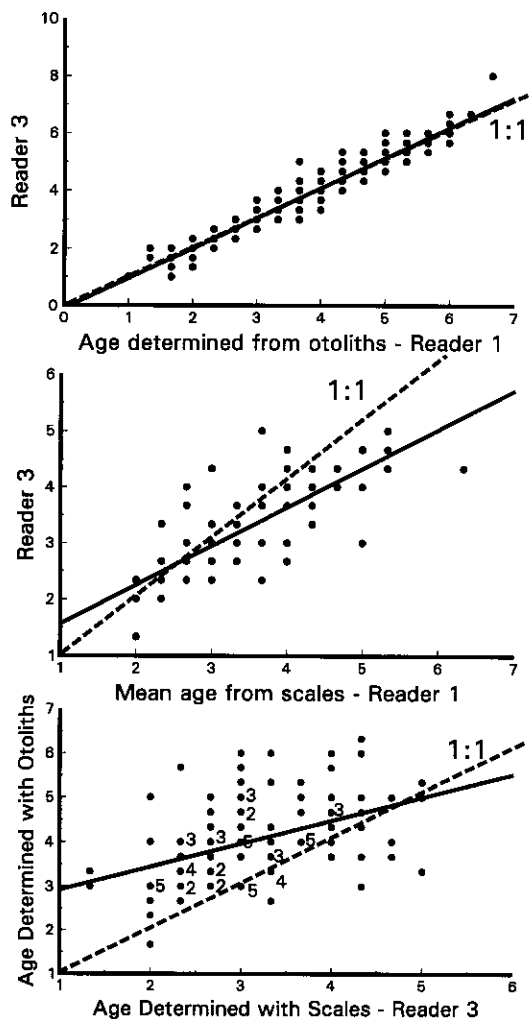


Figure 2. Age bias plots developed from mean ages estimated from otoliths and scales by reader 1 and reader 3. These plots are representative of the relationships in age among all three readers.

Mean back-calculated lengths at specific ages were computed based on otoliths (Table 2). Hatchery fish of given ages estimated from otoliths had significantly greater mean lengths than wild fish at all but age six (Table 3). Scales showed similar results (Table 3). The differences were largest at age one and declined as the fish grew older.

Mean back-calculated lengths at specific ages based on otoliths were less than those based on scales. Back-calculated lengths based on otoliths

were 59 mm less than those based on scales at age one for wild fish, and they continued to be significantly less ( $P < 0.02$ ) through age five, although the magnitude of the difference declined with increasing age. At age six there was no significant difference.

Estimates, based on otoliths, of mean ages of 18 YSC from the four streams stocked with hatchery fish revealed that 5- and 6-year-old fish were most abundant (Figure 3). However, the same fish were estimated to be 3- and 4-years old using scales. The known age of the hatchery fish was 6 years.

## Discussion

Fisheries scientists use measures of growth, mortality, and age structure to describe fish populations and evaluate management actions. Accurate age data are required to determine these statistics (Schramm and Doerzbacher 1982). Previous studies have suggested that the scale method of age determination is inadequate in both coldwater and warmwater species (Moring et al. 1981; Wigtil 1984; Boxrucker 1986; Hubert et al. 1987; Kozel and Hubert 1987). Otoliths tend to yield higher and more accurate estimates of age for YSC than do scales. We found age estimates using otoliths to be more precise (lower mean CV) and less biased than were estimates based on scales, suggesting that otoliths provide a more reliable and accurate estimate of age of YSC in high-elevation streams. It is probable that YSC in the Greybull River drainage do not reach squamation length (42 mm, Brown and Bailey 1952) during their first summer because of late spawning and a short growing season in the high-elevation system (Alvord 1954; Laasko and Cope 1956; Bulkley 1961; Averett and MacPhee 1971; Lentsch and Griffith 1987), but this does not explain the magnitude of the difference between the mean age estimates using scales (3 to 4 years) and the known age of the fish (6 years).

Age bias plots (Figure 2) revealed results similar to the CV analysis. If estimated ages using scales were 1 year less than corresponding ages from otoliths (simply a function of inadequate length for squamation the first year), we would expect a strong linear relationship. However, while correlation coefficients were significant, they were not strong (all  $< 0.70$ ), suggesting that there were additional influences on estimates of age based

TABLE 2. Mean back calculated lengths (mm) at specific ages of Yellowstone cutthroat trout from 17 high-elevation streams in the Greybull River drainage, Wyoming, based on otoliths. Numbers in parentheses following stream names indicate location in drainage (Figure 1).

Stream	Age (yr)						N
	1	2	3	4	5	6	
Hatchery populations							
Anderson (1)	116	160	206	237	259	274	15
Cow (4)	118	167	251	278	293	304	16
Eleanor (7)	96	136	205	232	256	270	19
U.Greybull (8)	119	188	252	282	300	322	25
Wild populations							
Brown (2)	58	119	155	158			17
Chimney (3)	68	142	194	233	255	275	16
Deer (5)	73	148	205	240	256		14
Dundee (6)	69	139	197				2
L.Greybull (9)	68	132	196	226	294	319	10
Jack (10)	56	119	167	201			21
Mabel (11)	71	125	181	228	258		1
Md.F. Wood (12)	64	143	195	234	270		13
Wood (13)	72	149	215	252			21
Pickett (14)	85	157	192	212			17
S.F. Wood (15)	67	136	209	255			18
Warehouse (17)	61	128	186	225	244	266	18
Combination of hatchery and wild fish							
Venus (16)	102	155	222	257	283	268	16

TABLE 3. Mean back-calculated total lengths (mm) at specific ages for hatchery and wild populations of Yellowstone cutthroat trout from the Greybull River drainage, Wyoming, based on otoliths and scales.

Group	Age (yr)						N
	1	2	3	4	5	6	
Otoliths							
Wild	67	137	190	228	257	276	183
Hatchery	109	158	224	254	274	287	76
Scales							
Wild	126	184	233	261	291	324	80
Hatchery	149	214	266	284	292		20

on scales other than the lack of first year squamation. Reader error (Casselman 1987), scale erosion, and obscure annuli (Alvord 1954), may also lead to bias in age estimations based on scales.

Hatchery YSC were longer than wild YSC at young ages, but the difference declined as they grew older. Hatchery YSC may be expected to have faster growth while in a hatchery due to more optimal water temperature and greater food availability than is encountered by wild YSC in the Greybull River drainage. It is important to realize the implications

of these differences when assessing growth. If hatchery fish are included with wild fish in samples used for age and growth analysis, estimated length at specific ages will be greater than the actual lengths of wild fish (Table 3).

Comparisons with other cutthroat trout populations (Table 4) were made, but all previous age and growth estimates in published studies were based on scales. Back-calculated lengths at specific ages of wild YSC in the Greybull River drainage were low compared to other cutthroat trout

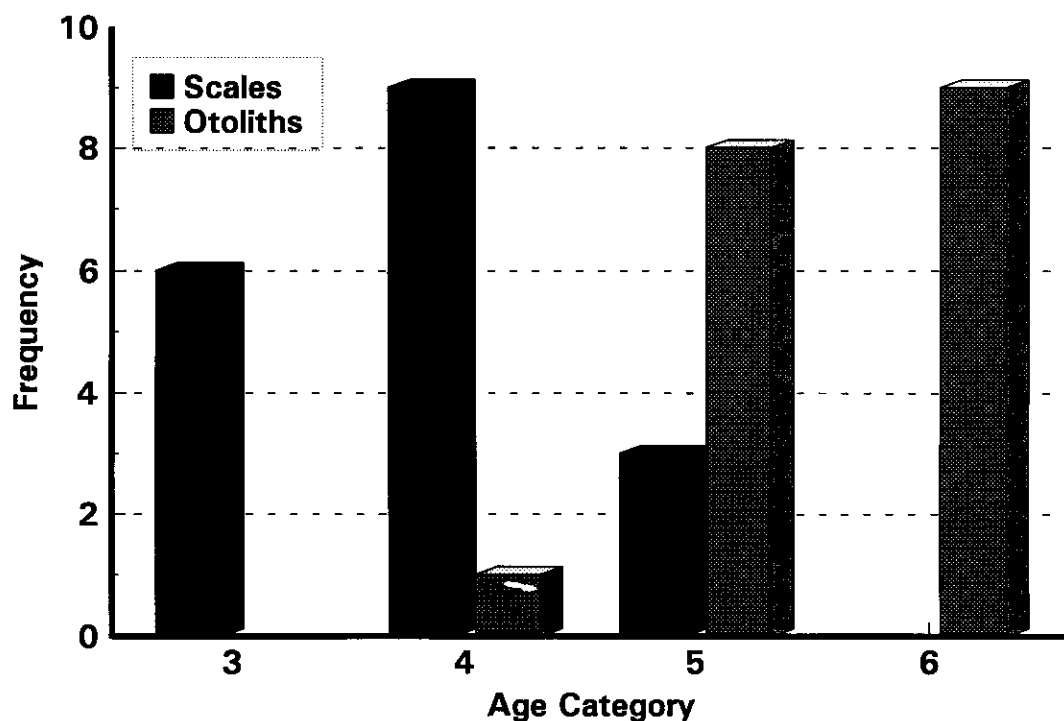


Figure 3. Frequencies of ages determined from otoliths and scales for samples from four study streams stocked with hatchery fish that were 6 years of age at the time of sampling and with no wild fish.

TABLE 4. Mean back-calculated total lengths(mm) at specific ages of cutthroat trout populations throughout the western United States based on scales, and estimates for wild Yellowstone cutthroat trout (YSC) from the Greybull River (GR) drainage, Wyoming, based on otoliths and scales.

Location	Age (yr)						Reference
	1	2	3	4	5	6	
GR scales	126	184	233	261	291	324	This study
GR otoliths	67	137	190	228	257	276	This study
Entire range of YSC	100	180	240	310	370	410	Varley and Gresswell (1988)
Yellowstone Lake	60	140	240	310	350	390	Gresswell(1995)
Montana (mean)	74	132	198	279	330		Carlander (1969)
N.F. Shoshone River, WY	67	134	229	304	375	403	Kent (1984)
Berry Creek, WY	124	186	256	301			Gulley and Hubert (1985)
Blackfoot River, ID	117	213	321	403	442	473	Thurrow (1982)
S.F. Snake River, ID	86	184	277	343	410	450	Moore (1984)
Sjhoberg, WY	91	147	218				Remmick (1981)
Bare Creek, WY	79	165	234				Remmick (1981)

populations. Back-calculated lengths based on scales were similar to those reported from other high-elevation cutthroat trout populations in Wyoming (Remmick 1981; Kent 1984). However, the slower growth that we describe based on otoliths is more likely to reflect actual growth in high-elevation mountain streams. Other species of trout in high-elevation montane streams will probably show similar results when aged using otoliths instead of scales.

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