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SOURCES OF VARIATION IN COUNTS OF MERISTIC FEATURES OF YELLOWSTONE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI BOUVIERI*)

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ABSTRACT.—We determined variability in counts of meristic features (pyloric caecae, vertebrae, pelvic fin rays, gillrakers, basibranchial teeth, scales above the lateral line, and scales in the lateral series) of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) by 3 independent readers, by the same reader on 3 different occasions, and among fish from 12 sampling sites within a 650-km^2 watershed. Genetic purity of the cutthroat trout was determined by electrophoretic analysis. Significant differences in meristic counts were observed among 3 readers and among sampling sites, but not among 3 occasions by a single reader. Scale counts were within the reported range for Yellowstone cutthroat trout, but counts of other structures (pyloric caecae, gillrakers, vertebrae) were as similar to rainbow trout as to Yellowstone cutthroat trout. Meristic counts identified the fish as cutthroat trout; however, variation among readers and sampling sites, as well as within the species, limits their use when identifying genetically pure cutthroat trout or assessing possible integration with rainbow trout.

Key words: meristic counts, Yellowstone cutthroat trout, meristic variation, genetics, rainbow trout, conservation biology.

Hybridization of native cutthroat trout (*Oncorhynchus clarki*) with introduced rainbow trout (*O. mykiss*) has contributed to the decline of cutthroat trout in the western United States (Allendorf and Leary 1988, Gresswell 1988, Behnke 1992). An important initial step toward restoration or preservation of native cutthroat trout populations is reliable identification of genetically pure populations (Rinne 1985, Leary et al. 1989).

Meristic features, such as fin ray or vertebrae counts, have been used to identify hybridization among species of trout. The technique assumes that hybrids are intermediate to parental taxa and have increased morphological variance (Leary et al. 1985, 1991, Marnell et al. 1987). This assumption is not always valid and meristic comparisons can provide misleading taxonomic information (Leary et al. 1984, 1985, Currens et al. 1989). Environmental influences and observer error are 2 factors that can lead to variation in meristic counts for a species among sampling sites (Currens et al. 1989, Leary et al. 1991, Hubert and Alexander 1995). Even though more definitive biochemical methods have been developed (Leary et al. 1987, 1989, Nielsen 1995), biologists continue to use meristic features to assess genetic purity of cutthroat trout populations (Loudenslager and Gall 1980, Rinne 1985, Behnke 1992).

Protein electrophoresis is a reliable method of determining genetic status of trout populations (Marnell et al. 1987, Leary et al. 1989, Nielsen 1995). Electrophoresis provides data on allelic frequencies at genetic loci for different populations (Avise 1974). Hybridization can be determined when allele frequencies unusual for a particular species are found at several diagnostic loci that occur between taxa (Ayala and Powell 1972, Leary et al. 1989). For example, Yellowstone cutthroat trout (*O. c. bouvieri*) can be differentiated from rainbow trout using alleles at 10 diagnostic loci (R. Leary, University of Montana, personal communication).

If this procedure is valid, managers could save considerable time and money using meristic features instead of biochemical analysis to assess genetic purity of cutthroat trout. However, unless variation in meristic counts is minimal among readers or sampling sites, the usefulness of meristic features in adequately assessing genetic purity will be limited. The objectives of this study were to determine variability in counts of meristic features (1) among

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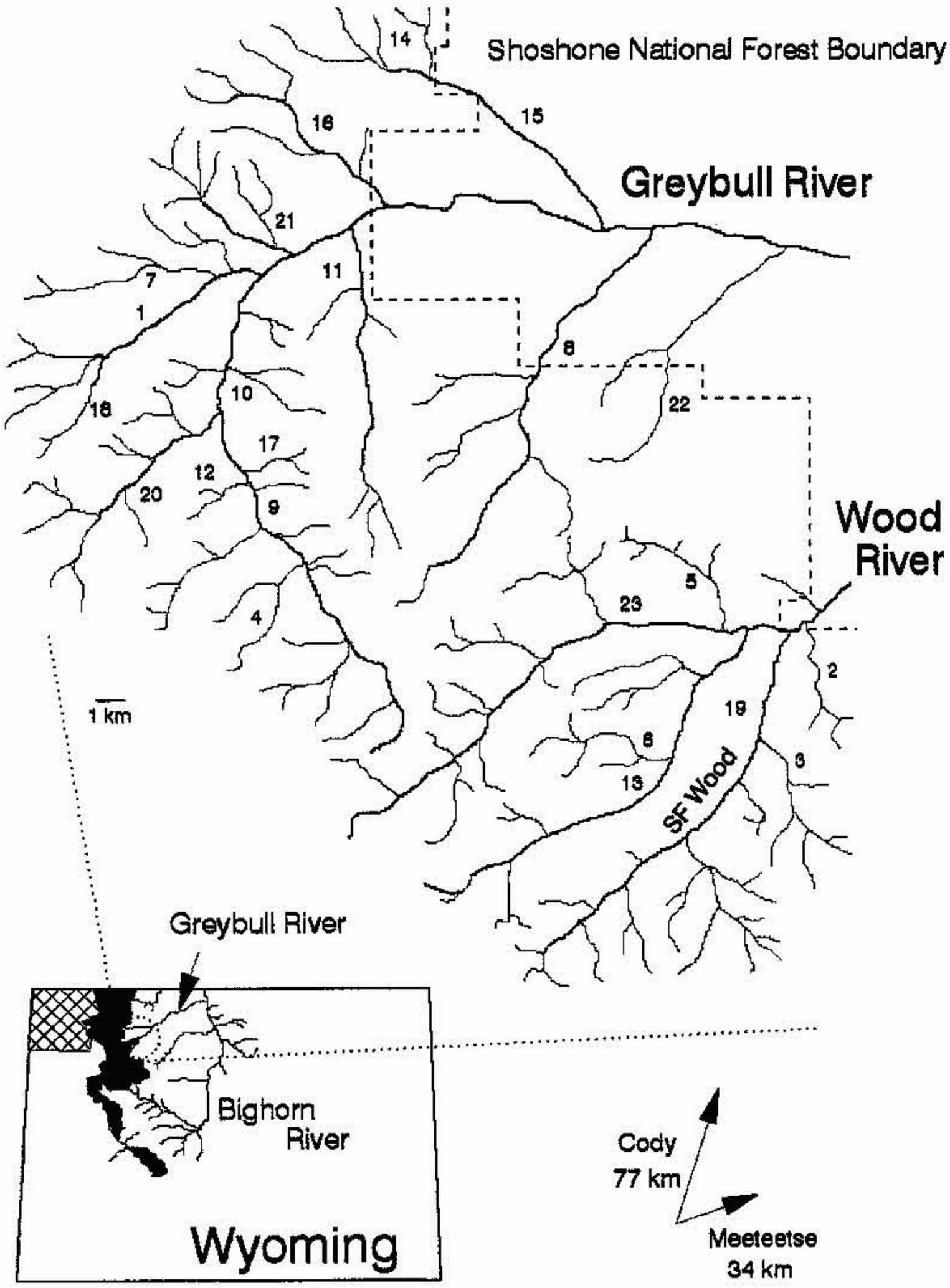

Fig. 1. Map of Wyoming showing the location of the Greybull River drainage. Sites where cutthroat trout were sampled are numbered in reference to Table 1.

independent readers, (2) among counts by a single reader, and (3) among sampling sites within a moderate-sized watershed (650 km^2).

STUDY AREA

The Greybull River drains 2900 km^2 of the eastern Absaroka Mountain Range in northwestern Wyoming. The study area includes that portion of the Greybull River drainage within the Shoshone National Forest (Fig. 1). A total of 56 perennial tributaries (355 km of total stream length) occur in the 650-km^2 headwater drainage.

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 area range from 2300 to 3050 m above mean sea level.

The Greybull River, within the historic range of Yellowstone cutthroat trout (Behnke 1992), is currently managed by the Wyoming Game and Fish Department as a sport fishery for native cutthroat trout and mountain whitefish (*Prosopium williamsoni*). Nonnative brook trout (*Salvelinus fontinalus*), finespotted cutthroat

TABLE 1. Streams containing cutthroat trout in the Greybull River drainage, number of fish collected, and sample sizes from each used for meristic counts and analysis. Genetic status indicated by pure Yellowstone cutthroat trout (P) or potential finespotted cutthroat trout hybridization (FSC). Number preceding the stream name corresponds to sites in Figure 1.

Stream		Number of fish collected	Allozyme analysis	Counted by all readers	Counted by single reader
1	Anderson	15	15 (P)	5	14
2	Brown	17		10	16
3	Chimney	16			15
4	Cow	16			11
5	Deer	16		4	16
6	Dundee	2			
7	Eleanor	19			3
8	Francs Fork				
9	Upper Greybull	15			7
10	Lower Greybull	20	20 (P)	1	20
11	Jack	21	19 (FSC)		10
12	Mabel	2		2	2
13	MF Wood	15	15 (FSC)		3
14	NF Pickett				
15	Picket	17	19 (P)		4
16	Piney				
17	Red	4		4	4
18	SF Anderson				
19	SF Wood	18	15 (FSC)	8	
20	Venus	16		9	10
21	Warhouse	18			14
22	W Timber				
23	Wood	21	20 (FSC)	7	18

trout, and rainbow trout have been stocked in the system.

METHODS

Twenty-three streams in the Greybull River drainage were sampled with battery backpack electroshockers from June to September 1994. Cutthroat trout were collected from 1 site (12–20 fish) on each of 18 streams. For analysis purposes the upper and lower Greybull River sites were considered separately (Table 1). Fish were collected from the midpoint of the length of each stream in which cutthroat trout were found. A sample of eye, liver, and muscle tissue was removed from each fish, wrapped in aluminum foil, and frozen within 1 h in liquid nitrogen. The remainder of each specimen was preserved in 75% ethyl alcohol. Tissue samples from each fish were individually identified.

Frozen tissue samples from 7 of the 18 streams were sent to the Wild Trout and Salmon Genetics Lab (WTSGL) at the University of Montana, Missoula, for genetic analysis. The 7 sites were selected to represent fish distribution in the drainage (Table 1, Fig. 1). Also, they were close to locations where finespotted cutthroat trout and rainbow trout had been previously introduced in the drainage (Wyoming Game and Fish Department records). Protein electrophoresis (Allendorf and Phelps 1980, Leary et al. 1984, Perkins et al. 1993) was performed to detect each specimen's genetic characteristics at 45 loci in muscle, liver, or eye tissue. Allele frequencies at 10 diagnostic loci (Table 2) were evaluated to determine hybridization with rainbow trout. Additionally, the presence of the AK-1*333 allele was evaluated to detect possible finespotted cutthroat trout hybridization.

Seven meristic features were counted on the preserved cutthroat trout: (1) basibranchial teeth, (2) anterior gillrakers (upper and lower limb of the first branchial arch), (3) pelvic fin rays, (4) scales in the lateral series, (5) scales above the lateral line, (6) pyloric caecae, and (7) vertebrae (Marnell et al. 1987, Behnke 1992). Three independent readers (all fisheries biologists with training in anatomy and taxonomy of salmonids) counted each meristic structure on the same 50 cutthroat trout (≥ 150 mm total length) chosen randomly from 9 of the 18

TABLE 2. Alleles at the 10 diagnostic loci that distinguish Yellowstone cutthroat trout and rainbow trout along with the tissue needed for each. The most common allele existing at each loci is listed first.

Locus	Characteristic alleles		Tissue
	YSC	RBT	
SAAT-1*	165	100,0	Liver
CK-A2*	84	100	Muscle
CK-C1*	38	100,150,38	Eye
mIDHP-1*	75	100	Muscle
sIDHP-1*	71	100,114,71,40	Liver
sMEP-1*	90,100	100	Muscle
sMEP-2*	110	100,75	Liver
PEPA-1*	101	100,115	Eye
PEPB*	135	100	Eye
PGM-1*	null	100,null	Muscle

streams (Table 1) 3 different times to assess repeatability and variation of counts within and among individual readers. One reader counted the 7 meristic features on 125 additional cutthroat trout to determine mean counts for each structure and allow comparison among the 12 sampling sites where ≥ 5 fish were counted (Table 1). The initial count from this reader's original 50 fish was also included in the analysis, leading to a sample of 175 cutthroat trout.

All counts were done on the right side of each cutthroat trout. Scales in the lateral series were counted 2 scale rows above the lateral line starting at the opercle opening and continuing to the insertion of the caudal fin, while scales above the lateral line were counted from the anterior of the dorsal fin on a vertical diagonal down to the lateral line. Vertebrae were counted during dissection of the fish. Pyloric caecae were enumerated by stretching the stomach and counting caeca ends. Meristic features were counted under a dissecting microscope using 30X magnification and reflected light. Readers practiced the protocol and compared results to resolve procedural differences before initiation of counts. All fish were counted at similar times by each reader with several different cutthroat trout counted between subsequent counts.

Three-way analysis of variance (ANOVA) was used to assess differences in counts of meristic features among (1) readers, (2) readings by individual readers, and (3) sampling sites. The sampling site effect was then controlled for and a 2-way ANOVA was used. One-way ANOVA was used to compare counts among readers and sampling sites. Tukey's multiple comparison test was used to make pairwise comparisons if significant differences were found. Statistical analyses were performed using SPSS/PC+ (SPSS Inc. 1991). Significance was determined at $P \leq 0.05$ for all tests.

RESULTS AND DISCUSSION

Cutthroat trout were present in all 23 study streams. Electrophoretic analysis of fish from 7 streams found no genes at diagnostic loci that identify rainbow trout (Table 2). Because genetic samples were collected from sites most likely to contain rainbow trout alleles (e.g., streams stocked with rainbow trout), we considered all trout in the drainage to be pure cutthroat trout.

The AK-1*333 allele is common among finespotted cutthroat trout in the Snake River drainage and was detected in 4 of the 7 samples (Table 1). This allele, while not unique to finespotted cutthroat trout, is rare in Yellowstone cutthroat trout populations outside the Snake River drainage; its presence indicates possible integration with finespotted cutthroat trout. An ANOVA showed no consistent difference in counts for any of the 7 meristic features between fish from sites potentially hybridized with finespotted cutthroat trout and those considered pure Yellowstone cutthroat trout. Additionally, Behnke (1992) stated that meristic counts of finespotted and Yellowstone cutthroat trout are indistinguishable, and there is considerable debate as to whether finespotted cutthroat trout are a formal subspecies. Therefore, we did not differentiate between finespotted and Yellowstone cutthroat trout in our analysis.

No significant differences among counts by the same reader for any meristic feature were observed. All 3 readers had high agreement among multiple counts for each structure (Table 3).

Significant differences in mean counts among different readers were observed for all structures except gillrakers (Tables 4, 5). All 3 readers had significantly different mean counts of pyloric caecae, pelvic fin rays, and scales above the lateral line, while at least 1 reader was significantly different from the other 2 readers in mean counts of vertebrae, basibranchial teeth, and scales in the lateral series. Hubert and Alexander (1995) also found poor agreement

TABLE 3. Significance values for differences in mean meristic counts among 3 readers (RDR), 3 readings by individual readers (RUN), and sampling site (SITE).

	Main effects			Interactions			
Structure	RDR	RUN	SITE	RDR×RUN	RDR×SITE	RUN×SITE	RDR×RUN×SITE
Pyloric caecae	0.000	0.903	0.000	1.000	0.000	1.000	1.000
Vertebrae	0.000	0.819	0.061	0.757	0.047	0.997	1.000
Pelvic fin rays	0.000	0.996	0.012	0.794	0.000	1.000	1.000
Gillrakers	0.765	0.356	0.244	0.352	0.045	0.098	0.051
Basibranchial teeth	0.448	0.945	0.000	0.952	0.323	1.000	1.000
Scales in lateral series	0.000	0.939	0.000	0.989	0.000	1.000	1.000
Scales above lateral line	0.000	0.986	0.000	1.000	0.000	1.000	1.000

TABLE 4. Significance values for the difference in mean meristic counts among 3 readers (READER) and among 3 readings by individual readers (RUN) at 5 sampling sites.

		Main effects		
Structure	Site	READER	RUN	Interaction
Pyloric caecae	Anderson	0.083	0.998	1.000
	Brown	0.000	0.993	0.808
	SF Wood	0.108	0.860	1.000
	Venus	0.227	0.932	0.972
	Wood	0.000	0.999	0.998
Vertebrae	Anderson	0.019	0.812	0.984
	Brown	0.000	0.618	0.561
	SF Wood	0.153	0.887	0.918
	Venus	0.016	0.886	0.969
	Wood	0.226	0.849	0.969
Pelvic fin rays	Anderson	0.000	0.802	0.924
	Brown	0.005	0.628	0.882
	SF Wood	0.000	0.880	0.924
	Venus	0.003	0.621	0.435
	Wood	0.000	1.000	1.000
Gillrakers	Anderson	0.596	1.000	1.000
	Brown	0.737	0.815	0.992
	SF Wood	0.001	0.871	0.492
	Venus	0.400	0.981	0.881
	Wood	0.055	0.938	0.880
Basibranchial teeth	Anderson	0.728	0.878	0.995
	Brown	0.000	0.683	0.902
	SF Wood	0.142	0.975	0.907
	Venus	0.064	0.889	0.990
	Wood	0.090	0.907	0.886
Scales in lateral series	Anderson	0.001	0.951	0.932
	Brown	0.000	0.860	0.818
	SF Wood	0.000	0.431	0.535
	Venus	0.000	0.879	0.905
	Wood	0.000	0.975	0.999
Scales above lateral line	Anderson	0.000	0.886	0.973
	Brown	0.000	0.888	0.843
	SF Wood	0.000	0.712	0.815
	Venus	0.000	0.885	0.885
	Wood	0.000	0.644	0.694

TABLE 5. Variation in mean meristic counts and standard deviations (in parentheses) of 3 readers. Means not significantly different indicated by bold (Tukey's $P \leq 0.05$).

Structure	Reader 1	Reader 2	Reader 3	P
Pyloric caecae	32.7 (6.3)	36.9 (9.5)	41.0 (11.7)	<0.0001
Vertebrae	60.5 (1.6)	**59.5 (2.0)**	**59.3 (1.2)**	<0.0001
Pelvic fin rays	9.0 (0.4)	8.8 (0.4)	9.4 (0.6)	<0.0001
Gillrakers	**18.9 (1.6)**	**18.8 (1.3)**	**19.3 (10.8)**	0.83
Basibranchial teeth	**13.7 (4.2)**	15.3 (4.3)	**14.2 (4.2)**	0.003
Scales in lateral series	178.0 (14)	**187.5 (14)**	**187.4 (13)**	<0.0001
Scales above lateral line	44 (4.2)	56.4 (5.2)	42.5 (3.6)	<0.0001

TABLE 6. Mean meristic counts and standard deviations (in parentheses) for 175 fish by 1 reader with ranges among the 12 sample sites with ≥5 fish counted. A probability (P) of ≤0.05 indicates significant differences among sites.

Structure	Grand mean (s)	Range in means among sites	P
Pyloric caecae	42.29 (10.89)	29.9–51.4	<0.0001
Vertebrae	58.57 (1.39)	57.9–60.6	0.0002
Pelvic fin rays	9.23 (0.86)	9.0–9.9	0.0001
Gillrakers	18.80 (2.08)	17.8–19.9	0.0018
Basibranchial teeth	13.96 (5.45)	11.4–21.8	0.0025
Scales in lateral series	182.70 (14.77)	175.5–207.3	<0.0001
Scales above lateral line	40.39 (3.51)	37.1–45.5	0.0001

among readers when counting meristic features of rainbow trout.

Significant differences were observed in counts of meristic features among fish from 12 streams (Tables 3, 6). Meristic features may be environmentally controlled within specific areas or drainages (Barlow 1961, Rinne 1985, Currens et al. 1989), but environmental variables measured at each sampling site (elevation, gradient, and stream size) were not correlated with meristic counts in the Greybull River drainage (Kruse 1995).

Researchers have used meristic counts with varied success to identify subspecies of cutthroat trout (Loudenslager and Kitchen 1979, Loudenslager and Gall 1980, Marnell et al. 1987). Recent research has shown that meristic comparisons can provide potentially misleading information (Busack and Gall 1981, Leary et al. 1984, 1985) because meristic characteristics are often specific to localized populations (Behnke 1992) and are strongly influenced by genetic variation (Leary et al. 1991).

Behnke (1992) described typical meristic counts for Yellowstone cutthroat trout and rainbow trout (Table 7). Mean counts of meristic features of cutthroat trout from the Greybull River drainage (Tables 5, 6) were within ranges for Yellowstone cutthroat trout (Table 7); however, mean counts of pyloric caecae, vertebrae, and gillrakers were also within typical ranges for rainbow trout. Variation and similarity in counts of meristic features of Yellowstone cutthroat trout and rainbow trout make it difficult to determine species or hybrids using meristic counts alone. Only the presence of basibranchial teeth provided a distinction between the 2 species.

Variations among readers, and among sampling sites in a small geographic area, along with relatively wide ranges in counts for Yellowstone cutthroat trout and rainbow trout, make it difficult to differentiate these 2 species with certainty using commonly assessed meristic features (Table 7). Furthermore, it is unlikely that Yellowstone cutthroat trout × rainbow trout hybrids can be identified due to the extensive variation in counts.

TABLE 7. Ranges of meristic counts among species (YSC = Yellowstone cutthroat trout and RBT = rainbow trout), readers, and sampling sites.

Variable	YSC[a]		RBT[a]		Variation among readers[b]	Variation among sampling sites[c]
	Typical	Overall	Typical	Overall		
Pyloric caecae	35-43	25-50	37-55	30-70	33-41 (36.9)	30-51
Vertebrae	61-62	60-63	62-64	61-66	59-61 (59.8)	58-61
Pelvic fin rays	9	9-10	not reported		9 (9.0)	9-10
Gillrakers	19-20	17-23	19-21	17-24	18-21 (19.0)	18-20
Basibranchial teeth		present		present	14-16 (14.4)	11-22
Scales in lateral series	165-180	150-200	125-150	120-160	179-188 (184)	176-207
Scales above lateral line	45-50	40-55	30-32	26-35	42-57 (47.6)	37-46

[a]From Behnke (1992)
[b]Ranges are from the 9 readings taken for each structure with means in parentheses (3 readings by 3 readers).
[c]Ranges are from means for the 12 sampling sites that had ≥ 5 cutthroat trout (≥ 150 mm total length) counted (Table 6).

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