GENETIC PURITY, HABITAT, AND POPULATION CHARACTERISTICS OF YELLOWSTONE CUTTHROAT TROUT IN THE GREYBULL RIVER DRAINAGE, WYOMING

by

CARTER G. KRUSE

A thesis submitted to

the Department of Zoology and Physiology

and the Graduate School of The University of Wyoming

in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

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My goal was to evaluate genetic composition of cutthroat trout in the Greybull River drainage, Wyoming, and describe habitat and population characteristics of this population of high-elevation cutthroat trout. Electrophoretic analysis indicated that native Yellowstone cutthroat trout were hybridized with finespotted Snake River cutthroat trout, but no evidence of hybridization with rainbow trout was found. Meristic and morphological comparisons gave similar results. Wild cutthroat trout (Yellowstone x finespotted) occupy 45% of the perennial stream length in the drainage. High stream gradients and fish migration barriers exclude cutthroat trout from much of the drainage. Standing stocks are low in the drainage due habitat and environmental limitations. Cover (predominately pools formed by boulders) was the only habitat variable significantly related to cutthroat trout standing stocks. As expected in high-elevation environments, back-calculated lengths from otoliths indicated relatively slow growth.

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PREFACE

This thesis is organized into six chapters, each of which pertains to a different topic on Yellowstone cutthroat trout in the Greybull River drainage. Chapters 2,3,4, and 5 will be submitted independently for publication in scientific journals. Because each of these chapters represents an independent manuscript that must be able to stand alone, some redundancy occurs among chapters.

Throughout the thesis, finespotted (also known as Snake River) cutthroat trout were treated as a subspecies of cutthroat trout. The subspecies has not been formally described and subspecies classification has been questioned due the lack of geographical isolation from the typical large-spotted Yellowstone cutthroat trout. Conclusions and management considerations are addressed with this distinction of subspecies, but with full awareness of the controversy regarding taxonomy.

"We are charged with the perpetuation of native species insofar as possible. Historically, most of the reduction of cutthroat trout habitat area in the higher elevations has been traceable to our own stocking activities and those other conservation agencies such as the Forest Service. I refer mainly to introductions of brook and rainbow trout into cutthroat trout waters. I feel it is high time we make a listing of remaining pure cutthroat trout waters and set them aside as inviolate native trout waters regarding stocking." Wayne R. Seaman, Colorado Division of Wildlife, State Fishery Manager - March 9, 1964.

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INTRODUCTION

The cutthroat trout (*Oncorhynchus clarki)* attained the broadest distribution of any native trout species in North America. It is the only native trout species in Colorado, Wyoming, Utah, and Alberta, as well as the dominant native trout species in Nevada, Idaho, and Montana (Behnke 1988). At least 16 subspecies of cutthroat trout have been mentioned in the literature (Behnke 1979; Johnson 1987; Shiozawa and Williams 1988) with 14 subspecies generally recognized (Trotter 1987; Shiozawa and Williams 1988; Behnke 1992).

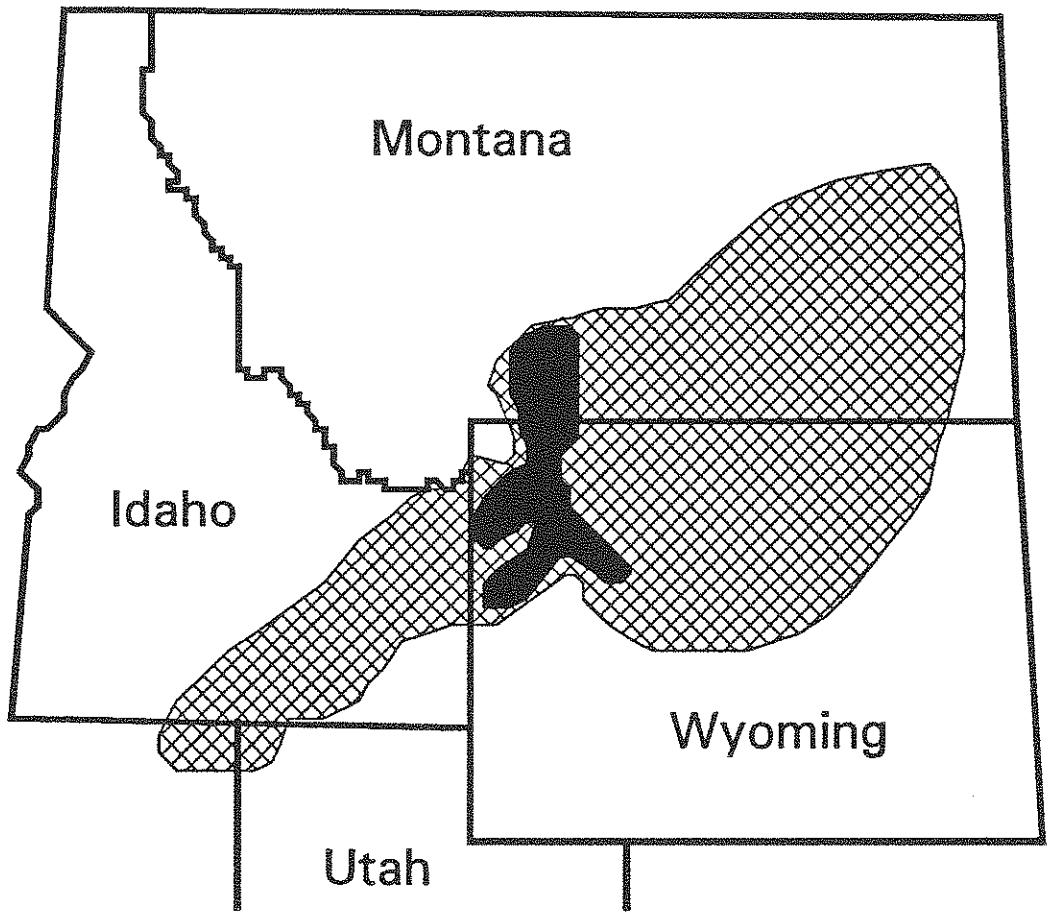
Historic distribution

The Yellowstone cutthroat trout (*O. c. bouvieri*) occupies the largest geographic distribution of the non-anadromous subspecies (Varley and Gresswell 1988). Yellowstone cutthroat trout were indigenous to the upper Columbia River and Yellowstone River drainages. However, since the last glacial period ended 12,000 years ago (Malde 1965; Gresswell 1995) and the invasion of redband trout (*O. mykiss gairdneri*) into the Columbia River basin, Yellowstone cutthroat trout have been isolated in the Snake River drainage above Shoshone Falls, Idaho, and the Yellowstone River drainage upstream from the mouth of the Tongue River in Montana (Varley and Gresswell 1988; Behnke 1992; Gresswell 1995; Figure 1). Since settlement by Europeans, distributions of genetically pure Yellowstone cutthroat trout have been reduced substantially (Behnke and Zarn 1976; Allendorf and Leary 1988; Varley and Gresswell 1988; Hedrick and Miller 1992; Gresswell 1995).

Factors affecting decline

Several studies have attempted to describe the magnitude of change in Yellowstone cutthroat trout from their range prior to settlement by Europeans and identify the factors responsible for change. Hanzel (1959) suggested that population declines and extirpations have been greatest in low-elevation, high-order streams with human activity, while remote mountain streams have enabled remnant populations of pure Yellowstone cutthroat trout to persist (Hanzel 1959; Behnke and Zarn 1976; Varley and Gresswell 1988). In the Bighorn National Forest of Wyoming only 66 km of a potential 1609 km (4%) of suitable habitat still support the subspecies (Kozel 1988). Pure Yellowstone cutthroat trout are believed to occupy only 8% of the original stream habitat in Montana (Hadley 1984). More recently, it was estimated that the subspecies' range, which historically included 4,800 km of streams in Montana and 15,100 km in Wyoming, has been reduced to 965 km and 4,700 km, respectively, in the two states (Gresswell 1995).

Figure 1. Probable past (pre-1880's, hatched area) and present (black area) ranges of Yellowstone cutthroat trout (Varley and Gresswell 1988).



Finally, Varley and Gresswell (1988) estimate that Yellowstone cutthroat trout remain in only 10% of nearly 24,000 km of original Yellowstone cutthroat trout stream habitat.

Hybridization - Introduction of exotic salmonids into the native range of Yellowstone cutthroat trout has been the most deleterious impact leading to the decline of the subspecies. Hybridization with the rainbow trout (*O. mykiss*; Loudenslager and Utter 1985; Gyllensten et al. 1985; Marnell et al. 1987; Allendorf and Leary 1988; Varley and Gresswell 1988) has destroyed the genetic integrity of native cutthroat trout populations and practically eliminated pure populations. Despite evolutionary diversity, cutthroat trout evolved apart from the redband trout and rainbow trout; thus, they lack isolating mechanisms that allow them to coexist without hybridizing. According to Hanzel (1959) rainbow trout were first introduced to inland waters west of the Continental Divide in 1891 and have been stocked extensively since that time.

Introductions of various cutthroat trout subspecies outside their native range also compromises genetic purity of native stocks. Snake River cutthroat trout have been introduced into many Yellowstone cutthroat trout waters; however, the extent of hybridization is unknown (Gresswell 1995).

Displacement - Brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) were both introduced to the West in the early 1890's and replaced cutthroat trout in much of their native range (Hanzel 1959). The displacement mechanisms are not entirely understood, but competition, displacement, differential angling mortalities, and exploitation have been suggested (Bjornn 1957; MacPhee 1966; Griffith 1974; Thurow et al. 1988; Varley and Gresswell 1988).

Habitat - Habitat loss or degradation has influenced the distribution of Yellowstone cutthroat trout. Dams, irrigation projects, mining, logging, agriculture, and energy development have impacted cutthroat trout (Behnke and Zarn 1976; Allendorf and Leary 1988; Thurow et al. 1988). These factors have not only decreased the availability of suitable habitat, but also favored displacement of native trout by brook trout and brown trout (Behnke and Zarn 1976; Behnke 1992). Anthropogenic impacts continue to degrade habitat (Gresswell 1995).

Management

Management agencies have begun programs to preserve and restore Yellowstone cutthroat trout (The Yellowstone Cutthroat Trout Working Group (YCTWG) 1994; Gresswell 1995). The American Fisheries Society has designated the Yellowstone cutthroat trout as a "Species of Special

Concern-Class A" (Johnson 1987) and this designation has been recognized by the states of Montana and Idaho. The U.S. Forest Service's (USFS) Northern and Rocky Mountain regions also consider the Yellowstone cutthroat trout a sensitive species (Gresswell 1995). Management agencies in Wyoming and Montana have jointly developed an interagency management guide formalizing and clarifying management strategies in the Yellowstone River basin (YCTWG 1994).

JUSTIFICATION

Maintenance and restoration of genetically pure populations of native trout is a goal of the WGFD and USFS (Leary et al. 1989; YCTWG 1994). Preservation of genetically unaltered populations to maintain native animal diversity is a priority.

Identification of pure, natural populations is the first step in a management program. Maintaining genetically pure populations and preventing the effects of reduced genetic variation (Allendorf and Leary 1988) is important to preservation and prevention of listing under the Endangered Species Act.

Since Wyoming is believed to have some of the few remnant populations of pure Yellowstone cutthroat trout (Varley and Gresswell 1988; Behnke 1992; YCTWG 1994), the WGFD has focused on the subspecies. Although their generalized distributions in Wyoming are known, it is not known where genetically pure populations occur. Identification of these locations is critical to future management and preservation of the subspecies in Wyoming, as well as to maintain and enhance sport fisheries in areas where the subspecies occurs.

The Yellowstone subspecies was the most widespread of the cutthroat trout subspecies in Wyoming, occurring in the Wind-Bighorn River, Clarks Fork, and the upper Snake River drainages (Thurow et al. 1988; Varley and Gresswell 1988; Behnke 1992). Fishery managers have identified several stream systems having high potential to contain genetically pure Yellowstone cutthroat trout, including: (1) Greybull-Wood River drainage; (2) North and South Forks of the Shoshone River; (3) Bighorn Mountains; and (4) the Clarks Fork of the Yellowstone River.

GOALS AND OBJECTIVES

The goal of this project was to identify locations of potentially pure Yellowstone cutthroat trout within the Greybull-Wood River drainage and describe habitat and population features. The WGFD identified the Greybull-Wood River drainage as having high potential to contain wild, genetically pure populations of Yellowstone cutthroat trout. Objectives were established to guide this work:

(1) determine the distribution of wild Yellowstone cutthroat trout with no evidence of hybridization

with rainbow trout or other cutthroat trout subspecies;

(2) assess the ability of meristic counts and morphometric comparisons to identify genetically pure Yellowstone cutthroat trout;

(3) determine the physical and biological factors that control standing stocks and allow pure, wild Yellowstone cutthroat trout to persist;

(4) describe general population features for wild Yellowstone cutthroat trout; and

(5) describe the management implications of these findings relative to the preservation of wild Yellowstone cutthroat trout.

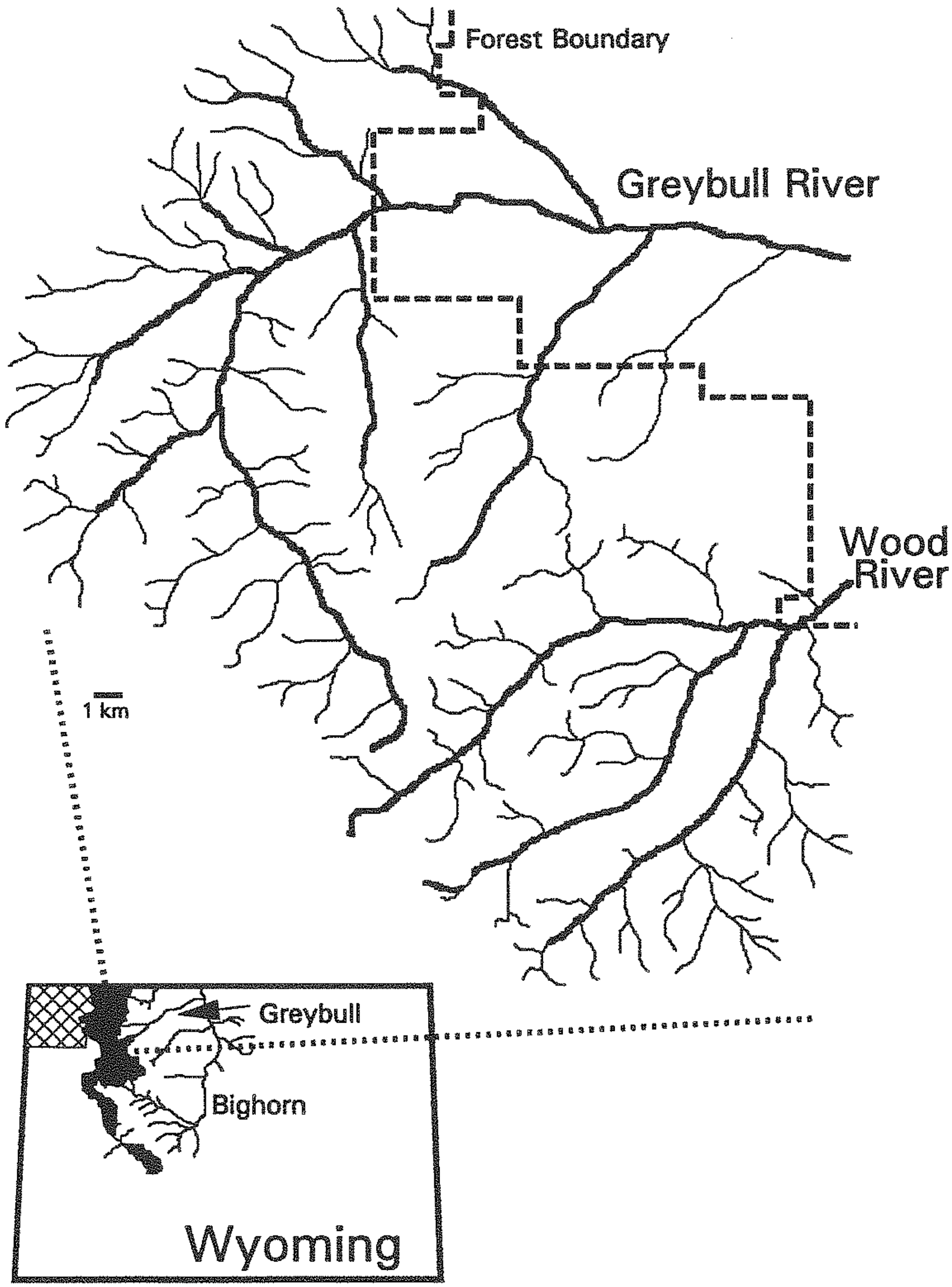
STUDY AREA

The Greybull River with its primary tributary the Wood River, is the third largest tributary to the Bighorn River. The Greybull River drains over 2975 km^2 of the eastern Absaroka Mountain Range in northwestern Wyoming, flowing 150 km before entering the Bighorn River near Greybull, Wyoming (Zafft and Annear 1992). The headwaters originate within the Absaroka Mountain Range, 64 km west of Meeteetse, Wyoming.

The study area (Figure 2) included the Greybull River drainage within the Shoshone National Forest, and four streams on Pitchfork Ranch Company lands adjacent to the national forest. A total of 56 major tributaries comprising 355 km of perennial streams (Appendix A) occurred 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 (Hansen and Glover 1973). Channel classification, according to Rosgen (1994), is predominately type A2, typical of high-gradient, straight, entrenched, mountain streams. Elevations throughout the study area range from 1800 m to 3700 m above mean sea level, with most streams between 2300 m and 3050 m (mean 2622 m). Stream gradient throughout the 56 tributary streams ranged

Figure 2. Map of Wyoming showing the Greybull River drainage and location of study area.



from 0.5 to 25% (mean 8.5%, considered steep; Kondolf et al. 1991; Rosgen 1994). Wetted stream width of the mainstem Greybull River during late summer varies from 3.5 m at the glacial headwaters to 25 m at the forest boundary, while the Wood River ranges from 2 to 18 m (Hansen and Glover 1973). Tributary streams ranged from 1.5 to 6.3 m (mean 4.1 m) in wetted width.

The Greybull River system has been developed for irrigation purposes (Zafft and Annear 1992), but water diversions first occur downstream from the study area. Water diversions, to fill Upper and Lower Sunshine Reservoirs, occur on both the Greybull and Wood rivers, 15 km downstream from the forest boundary (Zafft and Annear 1992). The hydrograph of streams in the study area follow a natural pattern. The winter discharge (February) of the Greybull River at Pitchfork Ranch is 0.51-0.85 m^3/s (Wyoming Water Resource Center, WWRC flow data) and mean peak spring flows (June) approach 56.6 m^3/s (Zafft and Annear 1992). Flows exceeding 226.5 m^3/s and as low as 0.28 m^3/s have been recorded (Hansen and Glover 1973; WWRC flow data).

Mean annual precipitation is 51 cm with 60% coming during spring (April-June). Snowmelt dominates the annual hydrograph and results in extremely high spring flows (Hansen and Grover 1973, Martner 1982, Zafft and Annear 1992).

The Rocky Mountains resulted from the Laramie orogeny nearly 75 million years ago. Large crustal disturbances led to intense volcanic activity (Keefer 1972) in early Eocene time (50-55 million years ago) resulting in the accumulation of the vast quantities of Absaroka volcanic rock (Nelson et al. 1980) which now compose most of the Absaroka Mountain Range. The landscape is steep and rugged, with uplifted peaks and deep valleys. Climatic and environmental weathering weakens porous breccia (hardened, angular volcanic deposits) and lava deposits contributing to massive debris torrents down mountain streams (Keefer 1972). Past geologic history, along with present rock deposits and topography, combine to make the Greybull River drainage geologically unstable.

These mountain streams have steep longitudinal profiles and low biologic productivity (Hansen and Grover 1973). Stream substrates and banks are predominately erosive, tuffaceous volcanic sediments and rubble (Hansen and Grover 1973; Zafft and Annear 1992). High spring flows in combination with steep gradients cause extensive streambank and bed erosion resulting in channels that shift regularly (Kent 1984; Zafft and Annear 1992), are strewn with large, angular rock (boulder and rubble; Hanzel 1959), are poorly defined, and provide limited fish habitat (Moore and Gregory 1989). Hansen and Grover (1973) noted a general lack of pool habitat in the Greybull River drainage.

The Greybull River within the study area is managed by the WGFD as a "sport" fishery for cutthroat trout and mountain whitefish (*Prosopium williamsoni*; Wiley 1992; Zafft and Annear 1992). Brook trout (*Salvelinus fontinalus*), longnose suckers (*Catostomus catostomus*), mountain suckers (*Catostomus platyrhynchus*), and longnose dace (*Rhinicthys cataractae*) are also present in the drainage (Yekel 1980).

The first recorded stockings of exotic salmonids in the Greybull River drainage occurred in 1915. Species stocked since that time have included: brook trout, unidentified subspecies of cutthroat trout, finespotted cutthroat trout, Yellowstone cutthroat trout, and rainbow trout (WGFD records). Finespotted cutthroat trout were stocked from 1972 to 1975 (Yekel 1980); thus, the potential for finespotted cutthroat trout to have occurred in tributaries of both the Wood and Greybull rivers exists. Yellowstone cutthroat trout have been stocked periodically since 1985 in several streams: Greybull River, Wood River, Anderson Creek, Venus Creek, Cow Creek, and Eleanor Creek (WGFD records).

Anthropogenic impacts on the Greybull River watershed have been relatively minor. Fishing pressure is low due to limited access to most areas (Yekel 1980). The Greybull River drainage, especially the Wood River drainage near Kirwin, has been prospected for minerals since the 1890's. Mining activity was highest between 1892 and 1907, with viable operations continued into the 1960's (Hansen and

Grover 1973). Due to limited human influence, the Greybull River drainage is considered to be essentially pristine.

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CHAPTER II:
CUTTHROAT TROUT DISTRIBUTIONS IN THE GREYBULL RIVER DRAINAGE, WYOMING

INTRODUCTION

The Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) inhabits a larger geographic range and is more abundant then any other cutthroat trout subspecies, with the exception of the coastal cutthroat trout (*O. c. clarki*; Varley and Gresswell 1988). The Yellowstone cutthroat trout (YSC) is indigenous to the Snake River upstream of Shoshone Falls, Idaho, and to the Yellowstone River upstream from the Tongue River, Montana (Behnke 1988; Varley and Gresswell 1988; Behnke 1992; Gresswell 1995). The distribution of YSC has declined to 8-10% of its range prior to settlement by Europeans (Hadley 1984; Kozel 1988; Varley and Gresswell 1988; Behnke 1992; YCTWG 1994).

Hybridization resulting from introductions of rainbow trout, nonnative cutthroat trout subspecies, or non-indigenous stocks of YSC trout has been the primary factor contributing to the reduction in YSC range (Loudenslager and Thorgaard 1979; Busack and Gall 1981; Campton and Utter 1985; Leary et al. 1984; Gresswell 1995). Competition with exotic fishes, as well as anthropogenic influences (logging, mining, agriculture, irrigation), have also reduced their range (Hanzel 1959; Allendorf and Leary 1988; Griffith 1988; Thurow et al. 1988; Gresswell 1995).

While generalized locations of YSC trout in Wyoming are known, accurate descriptions of their distributions are not available. Several areas have been identified by the WGFD as having high potential to contain genetically pure populations: (1) Greybull-Wood River drainage; (2) North and South Forks of the Shoshone River; (3) the Bighorn Mountains; and (4) Clarks Fork of the Yellowstone River.

Declines in YSC distributions have been most severe in high-order, low-elevation streams where human impacts are greatest (Hanzel 1959). Limited access to remote, high-elevation drainages, as well as public ownership of most of these systems, has contributed to protection of cutthroat trout populations and their habitat (Gresswell 1995). Hanzel (1959), Behnke and Zarn (1976), Scarnecchia and Bergersen (1986), and Varley and Gresswell (1988) noted that most genetically pure populations occur in high-altitude headwaters in remote areas. Additionally, Behnke (1992) suggested the cutthroat trout may have a selective advantage over nonnative trout in these areas because they may function better in cold environments.

High-elevation streams, where isolated populations of YSC appear to be competitively superior, tend to be narrow and shallow, have larger rock substrates and low water temperatures, and high gradients compared to downstream reaches (Platts 1979; Kozel et al. 1989). Abiotic factors, including gradient (Hocutt and Stauffer 1975; Kennedy and Strange 1982; Chisholm and Hubert 1986; Kozel and Hubert

1989; Kondolf et al. 1991), elevation (Fausch 1989; Kozel and Hubert 1989; Bozek and Hubert 1992), width and depth (Platts 1979; Bisson et al. 1988; Bozek and Hubert 1992; Heggenes et al. 1991), stream flow (Fausch and Northcote 1992; Rieman and McIntyre 1993), barriers (Rieman and McIntyre 1993), and bottom substrates (Moore and Gregory 1989; Kondolf et al. 1991) have been shown to affect abundance or distribution of trout.

Persistence of fish species in a stream system is influenced by the ability of the fish to successfully utilize the habitat for cover, acquire food, interact with other fish in the community, and reproduce (Bozek and Hubert 1992). Constraints imposed by physical habitat can limit species survival; thus, it is important to identify the abiotic factors that limit a species. Catastrophic events (Lamberti et al. 1991) and fish migration barriers (Stuber et al. 1988; Rieman and McIntyre 1993) can have major impacts on fish survival and distribution.

My goal was to evaluate the distribution of cutthroat trout in the Greybull River drainage and describe physical habitat characteristics that may limit them. My objectives were to: (1) determine the distribution of wild cutthroat trout in the Greybull River drainage; and (2) determine differences in physical habitat features between areas where cutthroat trout were present and absent in the Greybull River drainage.

METHODS

Study streams within the Greybull River drainage were selected using two criteria, availability of permanent stream flow based on U.S. Geological Survey (USGS) 1:24,000 topographic maps and potential to support a trout population (WGFD files). Streams were sampled from June to September 1994 using Smith-Root Model 12 POW battery-powered back-pack electroshockers.

At the confluence of each tributary with the Greybull or Wood river, sampling was initiated and progressed upstream at approximately 1 km intervals. At each location, a one-pass electrofishing run was performed over a 100-m stream reach. Stream reaches immediately below and above permanent barriers to fish migration (Stuber et al. 1988; Rieman and McIntyre 1993) were also sampled to assess barrier affects on fish presence. Barriers were defined as geologic structures at least 1.5 m in height (Stuber et al. 1988), dry stream reaches, or reaches of very high gradient or velocity. Sampling progressed upstream until trout were no longer present; an additional upstream site was sampled to ensure fish absence further upstream in the drainage.

The locations (latitude-longitude) and elevations (meters) of sampling sites and barriers to fish migration were identified with global positioning units and topographic maps. Barrier height (nearest 0.1 m), width

(0.1 m), and composition were recorded.

Wetted stream width (nearest 0.01 m) was measured perpendicular to stream flow at four transects spaced equally over the 100-m reach. Thalweg depths (0.01 m) and bankfull channel width (0.1 m) were measured at each transect. Channel-full banks were delineated according to the criteria of Lowham (1976). Gradient (%) was estimated with a clinometer over the entire 100-m reach. Substrate composition was visually estimated across three different transects spaced equally through the reach and categorized (percent composition) into four size categories: (1) boulder (> 30.5 cm diameter); (2) rubble (7.6-30.5 cm); (3) gravel (0.25-7.6 cm); and (4) sand and silt (< 0.25 cm; WGFD stream survey protocol). Mean wetted width to depth ratio was calculated for each reach (Kozel and Hubert 1989; Fausch and Northcote 1992; Rosgen 1994). Wetted width to bankfull channel width ratios were used as an index of the magnitude of flow fluctuation between spring high flow periods and late summer low flows. Stream lengths (kilometers) were measured with an electronic map wheel from 1:24,000 USGS topographic maps.

Sampling sites (151 on 56 streams) were grouped into four categories: (1) fish present, no barrier to migration; (2) fish present above downstream barrier(s) to migration; (3) fish absent, no downstream barrier to migration; and (4) fish absent above a barrier to fish migration. One-way analysis of variance (ANOVA) was used to test for significant differences in abiotic habitat features among the four categories. When significant differences were found, a Tukey multiple-comparison test was used to determine which categories were different. Least-squares linear-regression analysis was used to determine relations between habitat variables (Krebs 1989). Statistical analyses were performed using SPSS/PC+ (SPSS Inc. 1991). Significance was determined at $P \leq 0.05$ for all tests.

RESULTS

The distributions of all trout species were described using samples from 151 locations on 56 streams throughout the Greybull River drainage. Cutthroat trout were present in 15 of 33 (58 of 89 sites) Greybull River tributaries and 7 of 23 (31 of 62 sites) Wood River tributaries (Appendix A).

Brook trout were sampled in two of the 56 study streams, both within the Wood River drainage. Rainbow trout were not collected.

Habitat variables were assessed to determine differences among sites where fish were: (1) present with no downstream barrier to fish migration; (2) stocked fish were present upstream from a barrier to fish migration; (3) fish were absent upstream from a barrier; and (4) fish were absent with no downstream barrier. Cutthroat trout were assumed to be introduced stocks if they were found in areas originally barren of cutthroat trout but recently planted with YSC by WGFD (WGFD records). Elevations of the study sites ranged from 2097 to 3206 m, gradients from 1.0 to 25%, and wetted widths from 1.3 to 15.8 m. Wetted width to depth ratios were between 3.7 and 40.4, and wetted width to bankfull channel width ratios ranged from 0.07 to 0.74 (Table 1).

Cutthroat trout were found at one site with a gradient of 17% (Mabel Creek); however, no other site with cutthroat trout present, above or below fish migration barriers, had gradients higher than 9%. No fish were found at elevations above 3182 m. Mean width, width to depth and width to bankfull ratios, and substrate composition were highly variable among all sites sampled.

Mean elevation differed significantly among the four categories of fish presence. Mean elevation was significantly lower at sites where fish were present without downstream barriers compared to sites where fish were stocked above barriers or where fish were absent (Table 1). Elevation did not differ among sites void of cutthroat trout, with or without barrier affects, and sites with stocked cutthroat trout upstream from barriers.

Gradient was significantly higher at sites where fish were absent without influence from barriers to fish migration than both categories of fish absence.

Table 1. Physical habitat variables for the four categories of cutthroat trout presence. Lines represent mean values that did not differ significantly between categories (Tukey, $P \leq 0.05$).

Variable		Category of Fish Presence: Fish Present No Barrier	Fish Absent No Barrier	Fish Present Above Barrier	Fish Absent Barrier Present	P
Gradient	Mean	4.42	9.59	3.99	6.65	<0.001
	SD	2.88	5.59	1.65	2.92	
	Range	1-17	0.5-25	2-7.1	2-12.5	
	n	73	38	15	23	
Elevation	Mean	2437	2768	2774	2657	<0.001
	SD	216	197	111	237	
	Range	2096-3182	2356-3146	2569-2996	2321-3206	
	n	74	39	15	23	
Wetted Width	Mean	4.91	3.04	4.15	3.14	<0.001
	SD	2.85	1.26	1.41	1.5	
	Range	1.4-15.8	1.3-7.7	2.3-7.4	1.5-6.8	
	n	74	39	15	23	

Table 1. Continued.

Variable		Category of Fish Presence				
		Fish Present No Barrier	Fish Absent No Barrier	Fish Present Above Barrier	Fish Absent Barrier Present	P
Width\ Depth Ratio	Mean	17.6	13.5	16.3	16.6	0.017
	SD	5.8	4.0	5.8	7.6	
	Range	4.1-40.5	7.2-25.7	3.7-27.9	7.6-34.7	
	n	74	39	15	23	
Width\ Bankfull Ratio	Mean	0.25	0.22	0.23	0.24	0.844
	SD	0.15	0.14	0.12	0.16	
	Range	0.08-0.68	0.07-0.74	0.1-0.51	0.07-0.64	
	n	70	35	15	23	
Boulder	Mean	23.9	43.4	32.3	33.7	<0.001
	SD	22	25.4	21.7	21.5	
	Range	0-90	0-80	10-70	5-95	
	n	74	37	15	23	

Table 1. Continued.

Variable		Category of Fish Presence				P
		Fish Present No Barrier	Fish Absent No Barrier	Fish Present Above Barrier	Fish Absent Barrier Present	
Rubble	Mean	47.6	33.2	43.7	36.7	0.001
	SD	18.9	20.4	14.8	14.11	
	Range	0-95	0-90	25-70	0-60	
	n	74	37	15	23	
Gravel	Mean	23.5	19.6	16.5	23.9	0.325
	SD	16.2	18.7	10.4	14.9	
	Range	0-80	0-80	2-40	5-60	
	n	74	37	15	23	
Sand/Silt	Mean	5.0	3.5	6.3	5.7	0.281
	SD	5.7	5.8	5.1	5.0	
	Range	0-20	0-25	0-15	0-20	
	n	74	37	15	23	

Table 2. Pearson correlations between measured habitat variables. Significant relationships indicated in bold ($P \leq 0.05$).

	Gradient	Wetted Width	Width/ Depth	Width/ Bankfull	Boulder	Rubble	Gravel	Sand/Silt
Elevation	**0.44**	**-0.35**	**-0.42**	0.05	**0.47**	**-0.38**	**-0.29**	0.02
Gradient		**-0.50**	**-0.45**	0.13	**0.60**	**-0.50**	**-0.26**	**-0.18**
Wetted Width			**0.61**	**0.17**	**-0.30**	**0.30**	0.16	-0.16
Width/Depth				-0.03	**-0.39**	**0.29**	**0.25**	0.02
Width/Bankfull					**0.28**	-0.14	**-0.20**	**-0.17**
Boulder						**-0.70**	**-0.60**	**-0.27**
Rubble							-0.10	0.03
Gravel								0.03

Figure 1. Trout distributions in the Greybull River, Wyoming.

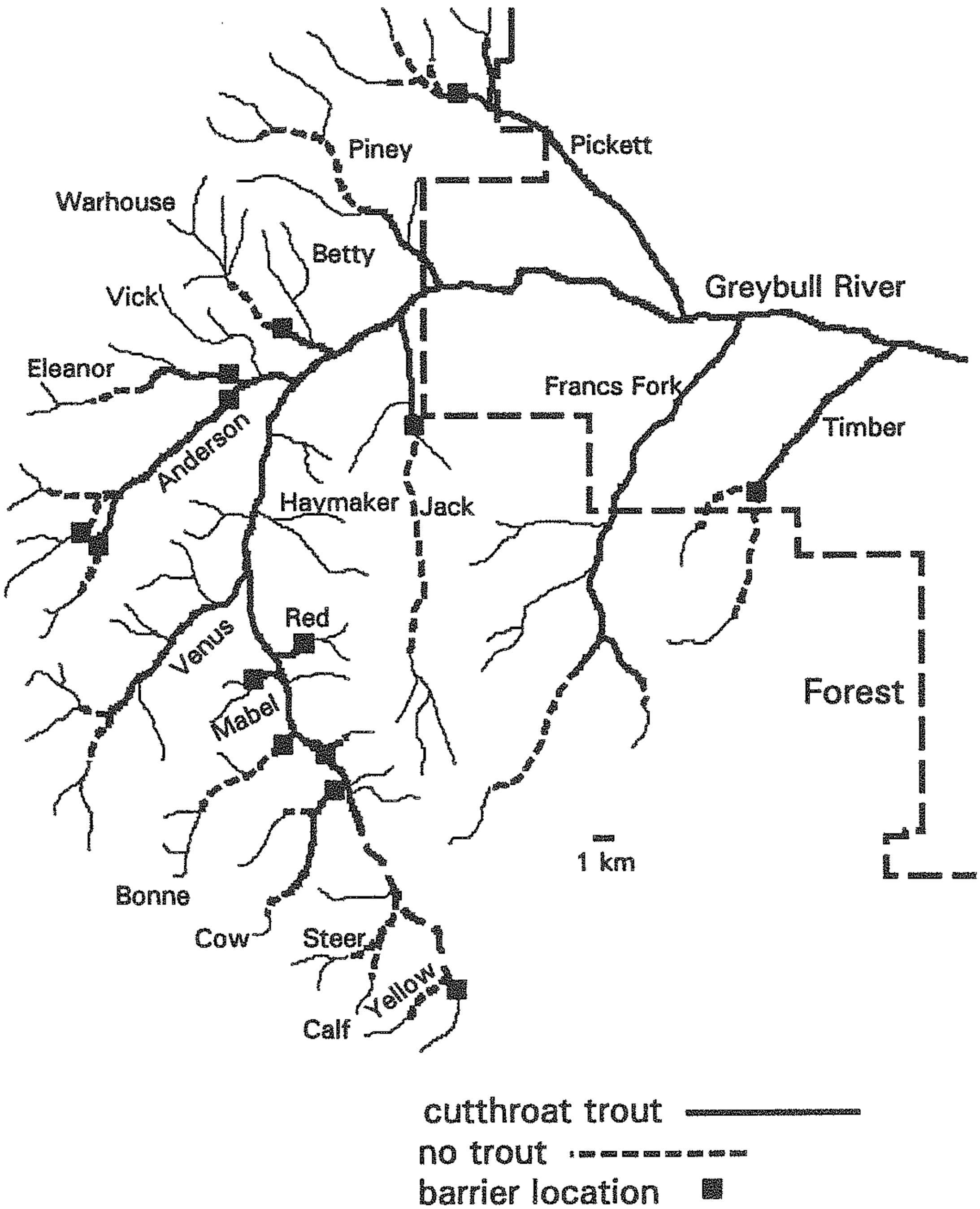
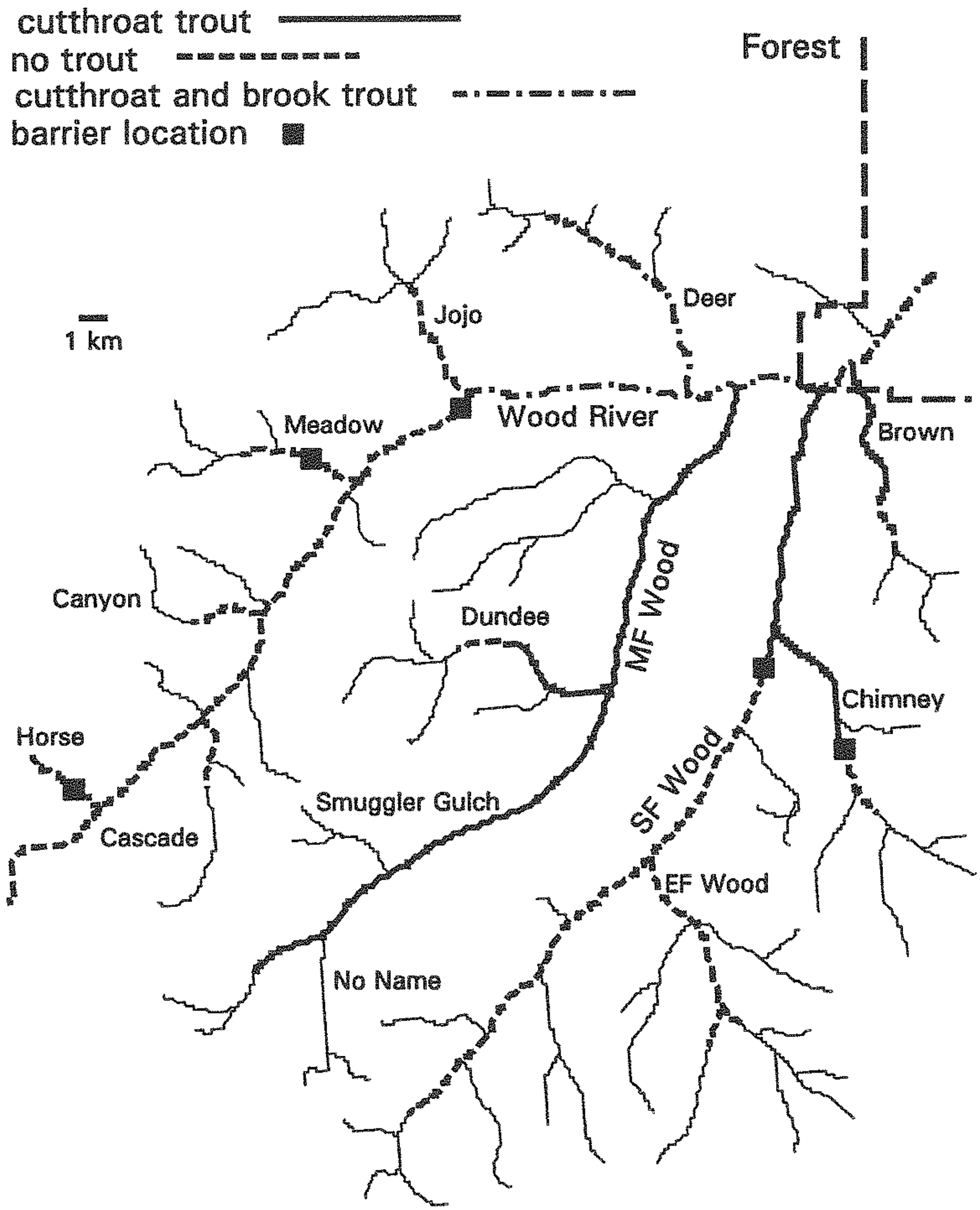


Figure 2. Trout distributions in the Wood River, Wyoming.



Additionally, sites with cutthroat trout above barriers to migration had significantly lower gradients than sites without fish not influenced by fish migration barriers. The proportion of sites occupied by cutthroat trout declined linearly with gradients from 0 to 9%; however, above gradients of 9% the proportion of sites occupied by cutthroat trout declined to zero, except for the one site of 17% (Figure 7).

Stream size, indicated by mean wetted width, was significantly greater at sites where fish were present with no barrier than at sites where fish were absent with or without a barrier (Table 1). Wetted width to depth ratios were only significantly different between sites with fish present and absent without barrier affects, while wetted width to bankfull channel ratios showed no significant difference among categories. Boulder and rubble substrate categories were significantly different between sites with fish and sites void of fish without barrier affects, but gravel and sand/silt substrates were not different among the four categories of fish presence. All habitat variables, except wetted width to bankfull channel width ratios, were significantly correlated with gradient (Table 2); indicating these variables are not independent predictors of fish distributions (autocorrelation).

Barriers to upstream migration of cutthroat trout were found on 17 of the 56 study streams (Figures 1 and 2).

Barriers appeared to have a profound impact on fish distributions throughout the drainage. Fish were present in 15 of the 17 streams blocked by barriers; in 11 of these 15, fish were present up to the base of the barrier and absent upstream. The four additional streams had recently been stocked with YSC above the barrier by the WGFD.

DISCUSSION

Distribution

Behnke (1992) and YCTWG (1994) report that the YSC was the only trout native to the Greybull River drainage. Since 1933 (Yekel 1980), brook trout, rainbow trout, and at least one nonnative subspecies of cutthroat trout (finespotted) has been introduced into the drainage (Appendix A).

Cutthroat trout were sampled from 116 km (49%) of 236 km of perennial streams in the Greybull River drainage and 44 km (37%) of 119 km of stream within the Wood River drainage. Within the entire Greybull River drainage, 45% (160 km) of the 355 total km of perennial streams contained cutthroat trout, while the remaining 55% lacked fish due to barriers to fish migration or inadequate habitat associated with stream gradients > 9%. Stocked YSC upstream from barriers occupy 20 km (12.5%) of the stream length where cutthroat trout were found.

Cutthroat trout distributions in the Greybull River drainage are probably similar to, or even greater than (due to stocking), historic levels. Cutthroat trout are present in every tributary that appears to be suitable for trout.

Brook trout, although shown to displace cutthroat trout through various mechanisms (Bjornn 1957; Varley and Gresswell 1988; Griffith 1974, 1988; Fausch 1989), were present in only two of 56 study streams, < 5% (12 km) of the length of perennial streams in the Greybull River drainage. In both streams they are sympatric with cutthroat trout. Although stocked in five streams (Francs Fork, Pickett, Timber, Blanchette, and JoJo Creeks) in the drainage from 1935 to 1949, brook trout remain only in Deer Creek and the Wood River and appear to have had minimal impact on YSC distribution in the Greybull River drainage.

Rainbow trout have been stocked in four study streams and in a fifth stream just downstream from the study area, but no rainbow trout were collected in 1994. The ability of rainbow trout to migrate throughout the system lends to the potential that hybridization with YSC may have occurred in the drainage.

In addition to five streams stocked with YSC since 1988, seven other streams have received introductions of unknown cutthroat trout subspecies. Both the Greybull and Wood rivers have been stocked with finespotted cutthroat trout. The ability of cutthroat trout to migrate (Heggenes et al. 1991) and the relatively short distances involved (Greybull = 33 km from headwaters to forest boundary, Wood =

24 km) suggest that YSC could have hybridized with other subspecies throughout the entire drainage.

Habitat

Abiotic habitat variables have been shown to affect fish distribution, abundance, and biomass of trout species in mountain streams (Kennedy and Strange 1982; Scarnecchia and Bergersen 1986; Kozel and Hubert 1989). Gradient is indicative of stream energy (Chisholm and Hubert 1986; Bozek and Hubert 1992; Rosgen 1994) and influences habitat (Kozel and Hubert 1989) and trout abundance (Chisholm and Hubert 1986). Bozek and Hubert (1992) showed that stream gradient was significantly related to cutthroat trout occurrence in high-elevation systems. Cutthroat trout occupied locations in streams with higher gradients than did brook trout, brown trout, and rainbow trout, but were not found in streams with gradients above 8%. Cutthroat trout distributions in the Greybull River drainage were also significantly influenced by gradient. Over 60% of the stream sites in the Greybull River basin had gradients considered steep (> 4%, Rosgen 1994).

Fish presence was influenced by reach gradient, with areas void of fish having mean gradients twice those where fish were found. Only one site where cutthroat trout were found, including those stocked above barriers, had a gradient > 9%, suggesting that gradient is limiting trout in streams with a gradient at or greater than 9%. Although several other habitat variables (elevation, wetted width, boulder and rubble substrates) were found to be significantly related to cutthroat trout distributions in the study area, gradient was probably the most influential habitat variable limiting fish distributions.

All study streams were above 2090 m and can be classified as high-elevation streams (Kozel and Hubert 1989). Bozek and Hubert (1992) found that elevation was a significant predictor of cutthroat trout locations within high-mountain streams of the Central Rocky mountains. Elevation can be categorized as a predictor of climate (Bozek and Hubert 1992) which influences several stages of the cutthroat trout life history (Gresswell 1995). If a portion of the life cycle is incompatible with climatic conditions resulting from elevational differences, elevation would directly impact fish distribution.

Elevation significantly influenced cutthroat trout distributions in the study streams; however, elevation was also positively correlated with gradient (Figure 3) and not an independent influence on trout distribution. Since gradient decreases with decreasing elevation (Figures 4,5), channel slope is probably the major variable influencing distributions.

Wetted width is a measure of stream size, but it is also related to climate and stream energy because larger

Figure 3. Relationship between elevation and gradient.

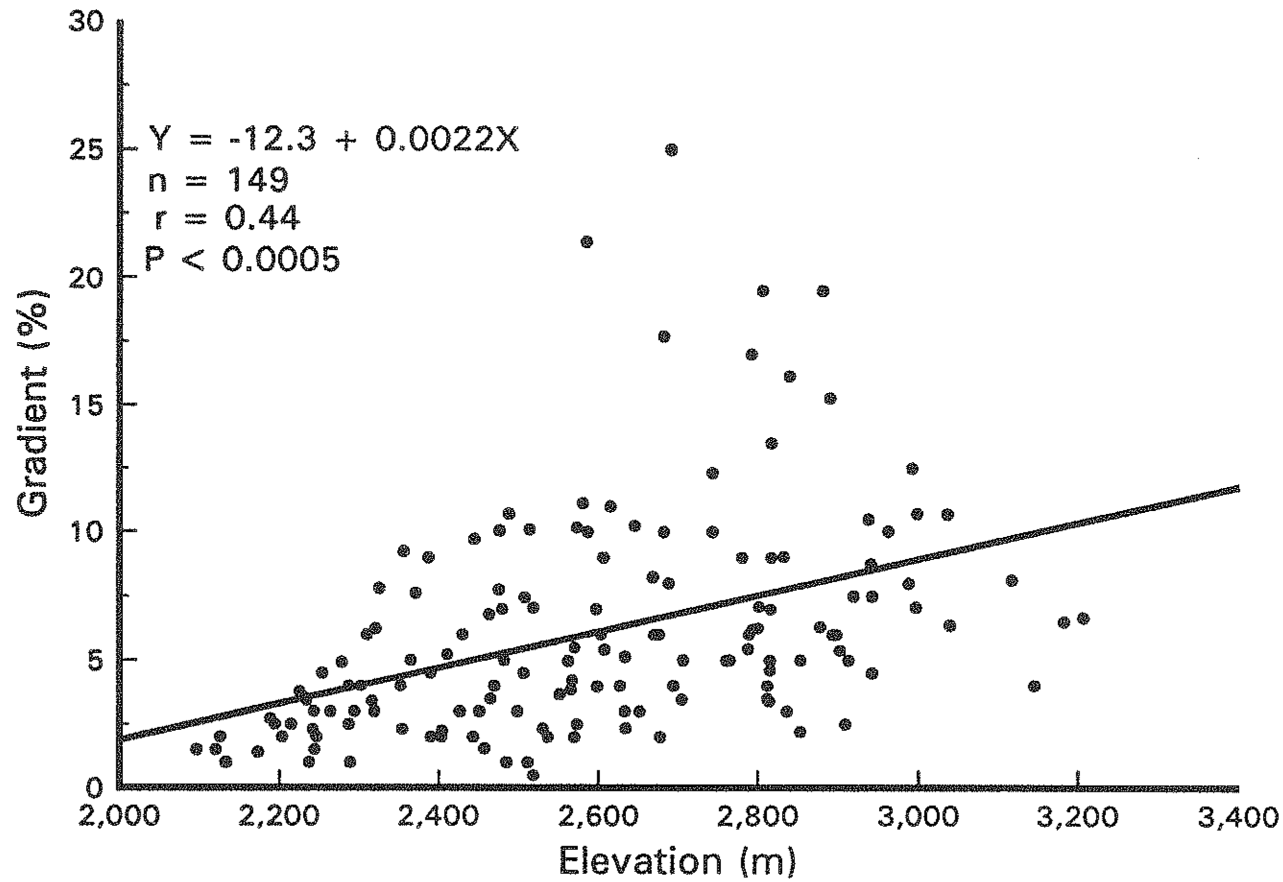


Figure 4. Longitudinal profile of Piney Creek.

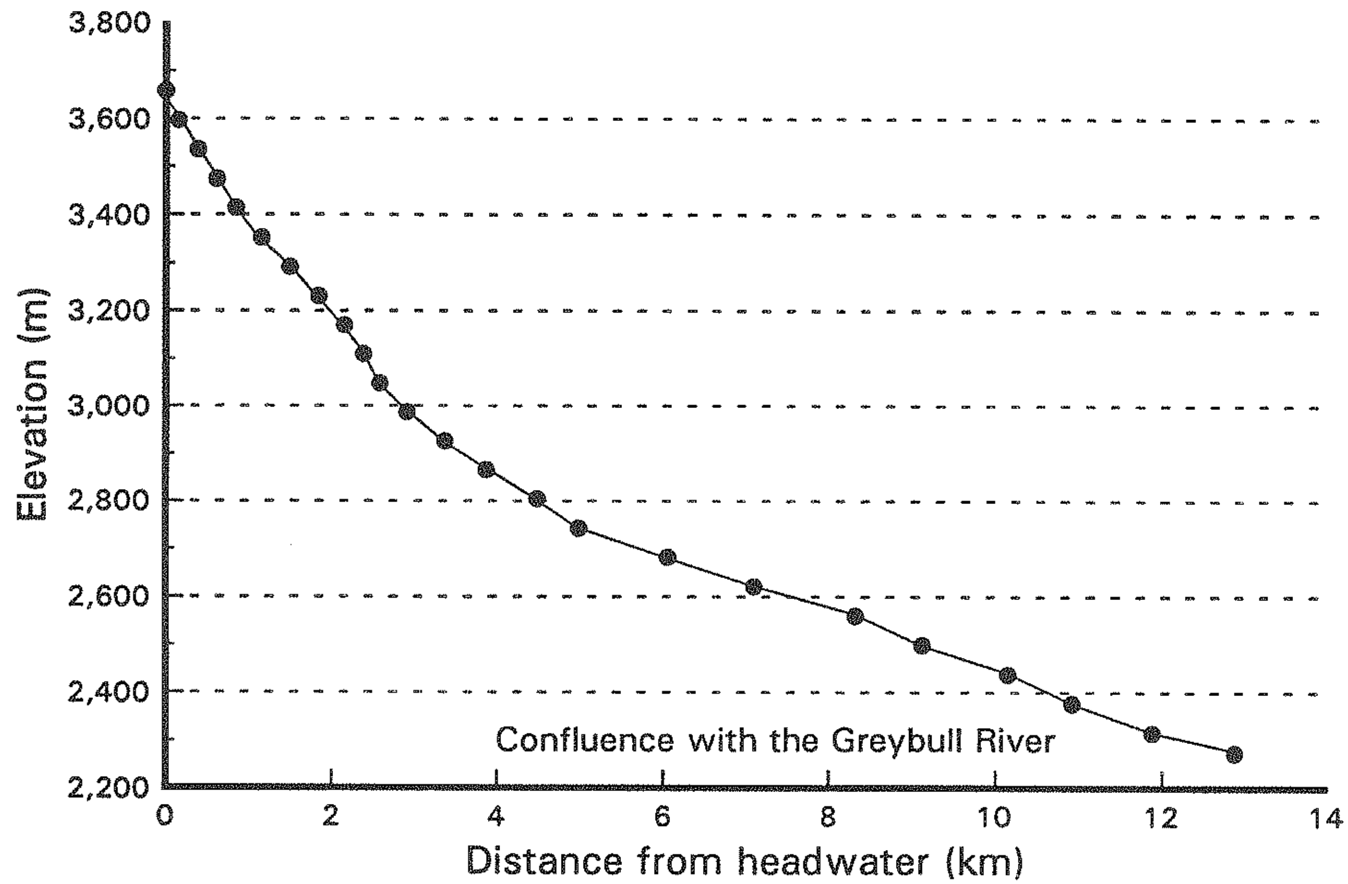


Figure 5. Longitudinal profile of Meadow Creek.

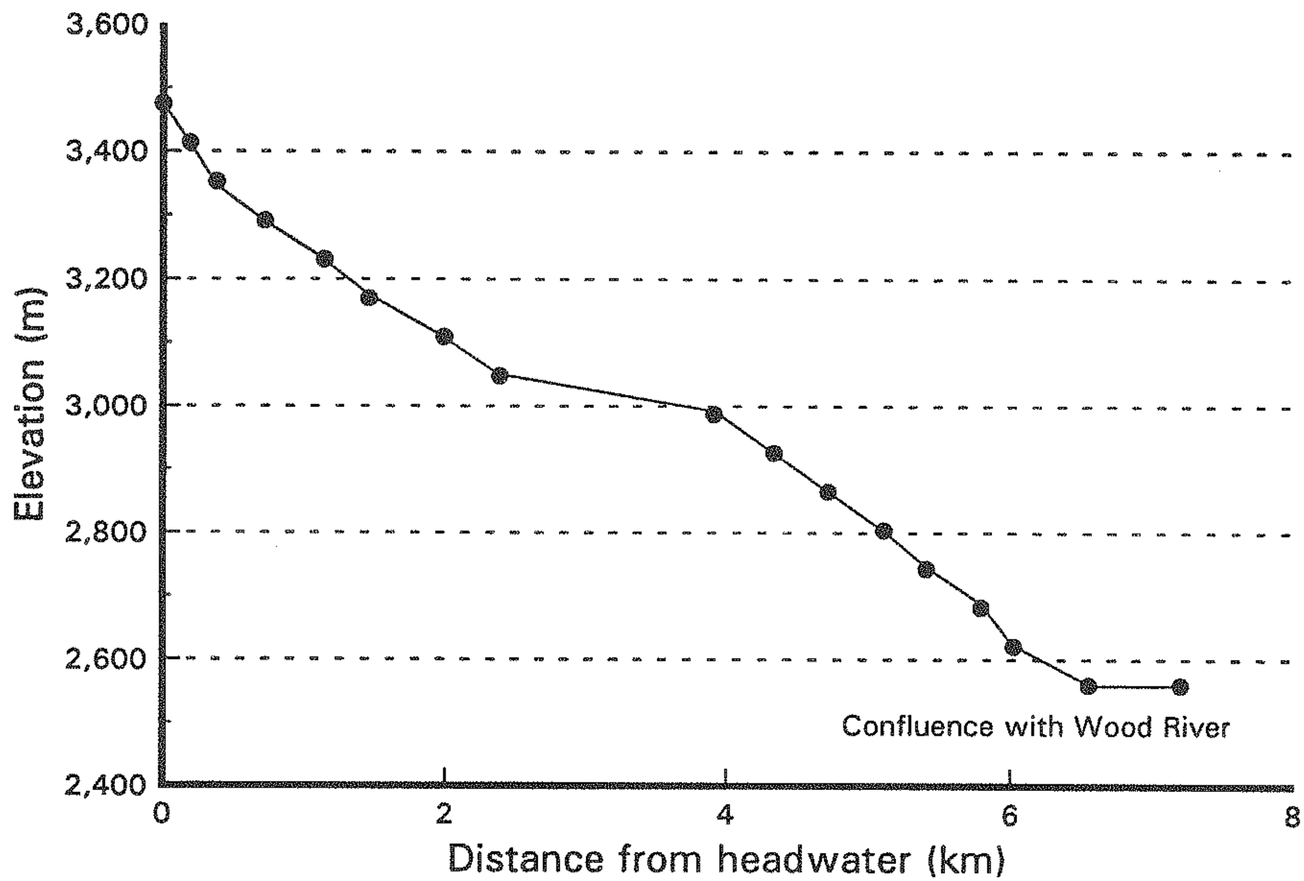


Figure 6. Relationship between substrate (boulder and rubble categories) and gradient.

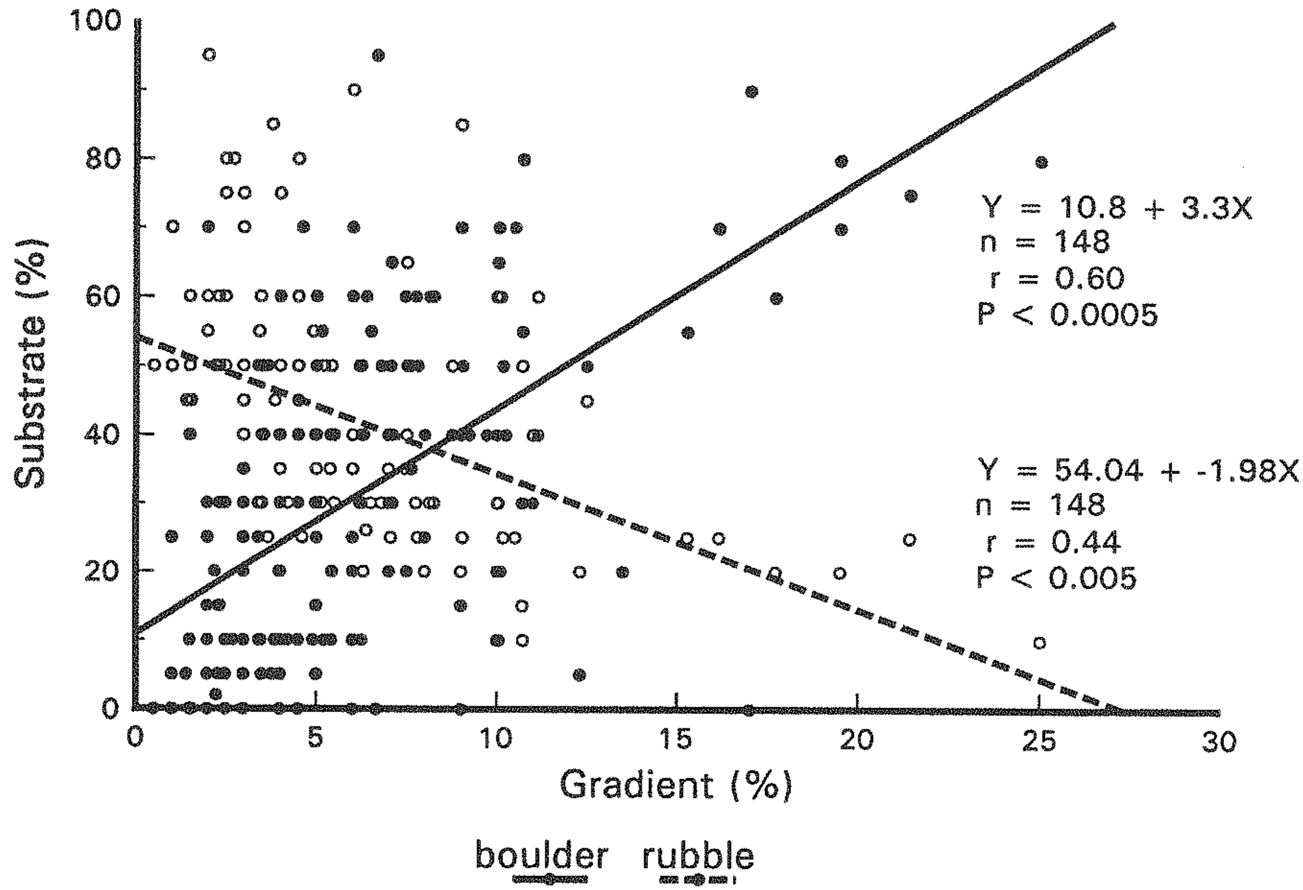
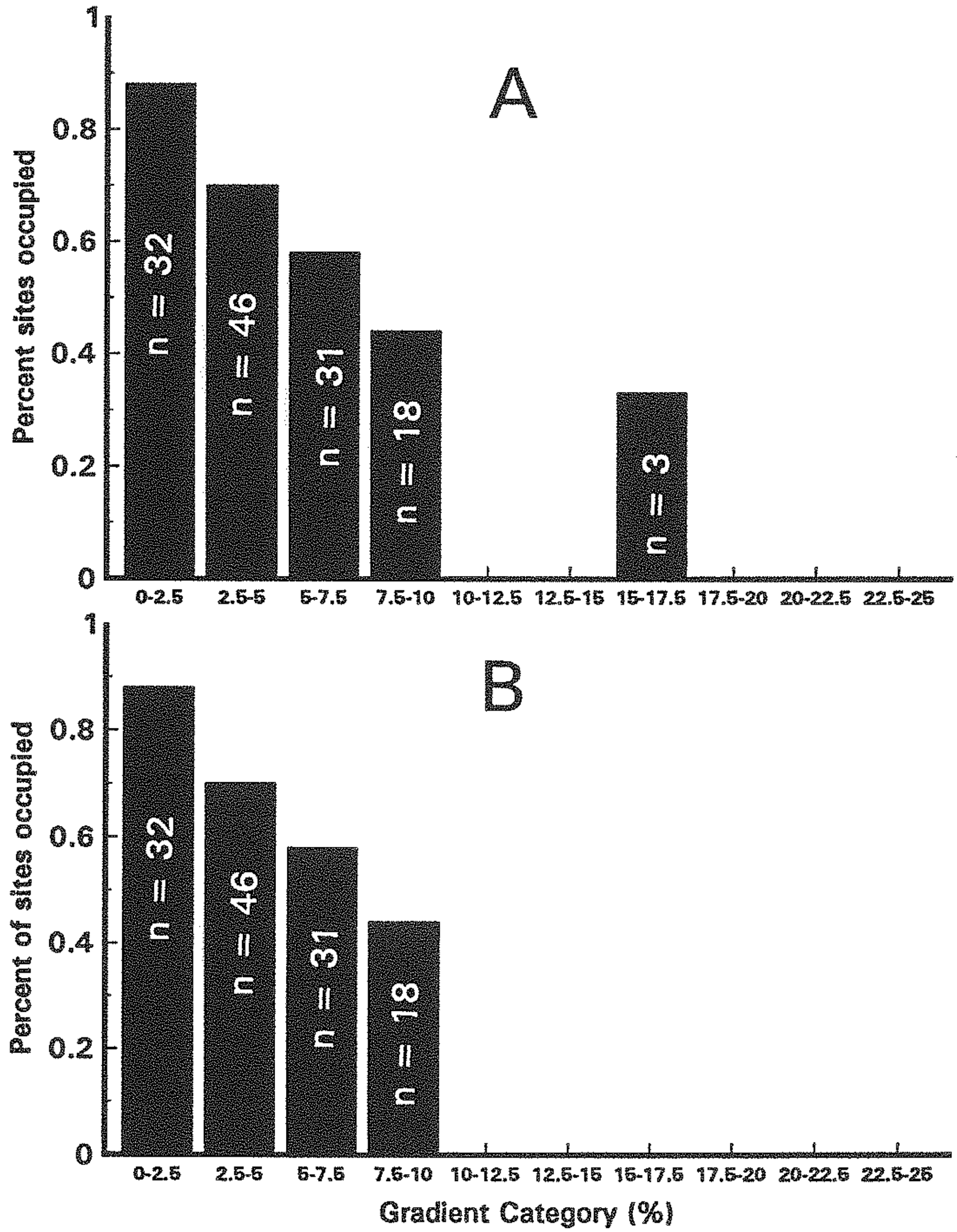


Figure 7. Proportion of gradient categories occupied by cutthroat trout including (A) and removing (B) the Mabel Creek site (17%).



streams tend to have lower gradients and warmer water temperatures (Bozek and Hubert 1992). Furthermore, stream size affects the availability of physical habitat types and abundance of trout species (Kennedy and Strange 1982; Chisholm and Hubert 1986; Bisson et al. 1988; Kozel and Hubert 1989; Heggenes et al. 1991a). Width to depth ratios combine two measures to provide an index of stream size (Rosgen 1994). Because bankfull flows have an average recurrence interval of 1.5-2.0 years (Mosley 1981), wetted width to bankfull channel width ratios, while not accurately describing the magnitude of annual flow fluctuations, give an idea of flow fluctuations over intervals of 1.5-2.0 years.

Within the study area, mean wetted width was significantly larger in areas where fish were present without barrier influence, than in both categories of fish absence. However, if the mean width of the Greybull River (15.6 m) is removed from the analysis, there is no longer a significant difference. Width to depth ratios were only significant between areas where fish were present and absent without barrier influence, while width to bankfull width ratios were similar in all four categories. Several studies have found stream size, discharge, and variation in discharge to be important factors influencing fish distributions and abundance (Minshall et al. 1983; Kozel et al. 1989; Bozek and Hubert 1992; Fausch and Northcote 1992);

however, in the Greybull River drainage, stream size and flow fluctuations do not appear to influence cutthroat trout distributions.

Substrate composition, similar to gradient, is indicative of stream energy. Substrate provides important stream habitat in the form of boulder pools, spawning habitat, and cover (Scarnecchia and Bergersen 1986; Kozel and Hubert 1989). Boulder and rubble substrates were significantly different among sites with and without fish; however, strong correlations with channel slope indicate that gradient is affecting substrate (Figure 6).

Barriers

Geologic barriers to fish migration influence fish distributions in the Greybull River drainage. Trout did not appear upstream of barriers, except the four streams where trout were stocked above them.

Catastrophic events may prohibit or limit cutthroat trout populations above barriers. It is unknown whether fish historically occupied areas above barriers in the drainage, or whether the barriers are relatively young geologically. Catastrophic events such as drought, floods, and debris torrents are common in the Greybull River drainage (Keefer 1972; Martner 1982; Lamberti et al. 1991). Flood flows, severe drought (30-100 year cycles in Bighorn basin; Martner 1982), and debris torrents (Lamberti et al.

1991) can locally decimate or extirpate trout populations. Swanson et al. (1987) suggests debris torrents are episodic events that may influence mountain streams every 50-200 yrs.

CONCLUSIONS

(1) Cutthroat trout occupy 45% of 355 km of perennial streams in the Greybull River drainage;

(2) Current distributions of cutthroat trout in the study area are probably similar to, or possibly greater than, historic distributions of cutthroat trout;

(3) Gradient is the physical habitat component that seems to have most influence on cutthroat trout distributions. Elevation and substrate composition also appear to influence fish presence; however, they are related to gradient and are not independent variables; and,

(4) Barriers influence cutthroat trout distributions by blocking upstream migration and preventing colonization of stream reaches above barriers.

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CHAPTER III: GENETICS OF CUTTHROAT TROUT IN THE GREYBULL RIVER DRAINAGE

INTRODUCTION

Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) historically inhabited most of the streams in the Absaroka Mountains of Wyoming (Thurow et al. 1988; Varley and Gresswell 1988; Behnke 1992; Gresswell 1995). In recent times, hybridization with rainbow trout (*Oncorhynchus mykiss*; Allendorf and Leary 1988; Varley and Gresswell 1988; Gresswell 1995), habitat loss (Varley and Gresswell 1988), and potential competition with introduced trout (Griffith 1974, 1988) have reduced the distribution of genetically pure Yellowstone cutthroat trout (YSC) in Wyoming.

Preservation of native cutthroat trout populations in Wyoming is the goal of both state (Wyoming Game and Fish Department, WGFD) and federal (U.S. Forest Service, USFS) agencies (Leary et al. 1989; Yellowstone Cutthroat Trout Working Group, YCTWG 1994). The initial step in implementing a restoration or preservation program is to identify stocks of genetically pure cutthroat trout native to various drainages (Leary et al. 1989).

In the past, morphological (such as spotting patterns and coloration) and meristic comparisons (such as fin ray or vertebrae counts) were used to identify hybridization among different species or subspecies of trout. The technique assumes that hybrids are morphologically intermediate to parental taxa and have increased morphological variance (Leary et al. 1985; Marnell et al. 1987; Allendorf and Leary 1988). Recent studies have shown that this assumption is not always valid and morphological and meristic comparisons can provide misleading information (Busack and Gall 1981; Leary et al. 1983, 1984, 1985). Regardless, several studies have used morphological and meristic characteristics (Binns 1977; Loudenslager and Gall 1980; Remmick 1981; Bisson et al. 1988; Behnke 1992) to determine genetic makeup of trout populations. Therefore, more definitive methods have been developed to identify hybrid trout.

Electrophoretic analysis of proteins is a powerful and reliable method of determining genetic status of trout populations (Leary et al. 1987, 1989; Marnell et al. 1987). Electrophoresis provides data on allelic frequencies at genetic loci for different populations (Avise 1974). Genetic composition of a population can be determined when complete differences in allele frequencies occur between taxa at several loci (Leary et al. 1989). Because of this, Ayala and Powell (1972) term the loci where differences exist between taxa as diagnostic loci. These loci are important in detecting hybridization (Leary et al. 1987).

Yellowstone cutthroat trout can be differentiated from rainbow trout by using eight loci commonly assayed in electrophoretic analysis. However, Leary et al. (1987) found no diagnostic loci among YSC and finespotted cutthroat trout from within the Snake River drainage. Recently, Robb Leary (Wild Trout and Salmon Genetics Laboratory (WTSGL), University of Montana, Missoula, personal communication) has been able to differentiate between finespotted cutthroat trout from within the Snake River drainage and YSC native to streams outside of the Snake River drainage.

Because identification of genetic purity is critical to management of cutthroat trout in the Greybull River drainage, the goal of this project was to determine if fish within the Greybull River drainage were genetically pure YSC. My objectives were to: (1) determine the genetic purity of cutthroat trout within the Greybull River drainage; (2) assess the ability of meristic counts to identify hybridization of YSC with rainbow trout or other cutthroat trout subspecies; and (3) determine the amount of variation within and among readers when counting meristic structures. Evaluating meristic counts and morphometric comparisons as methods of assessing genetic purity of cutthroat trout is important. If they are valid techniques, managers could save considerable time and money using meristics instead of electrophoretic analysis. However, unless variation in meristic counts is minimal among readers, use of meristics to assess genetic purity is limited.

METHODS

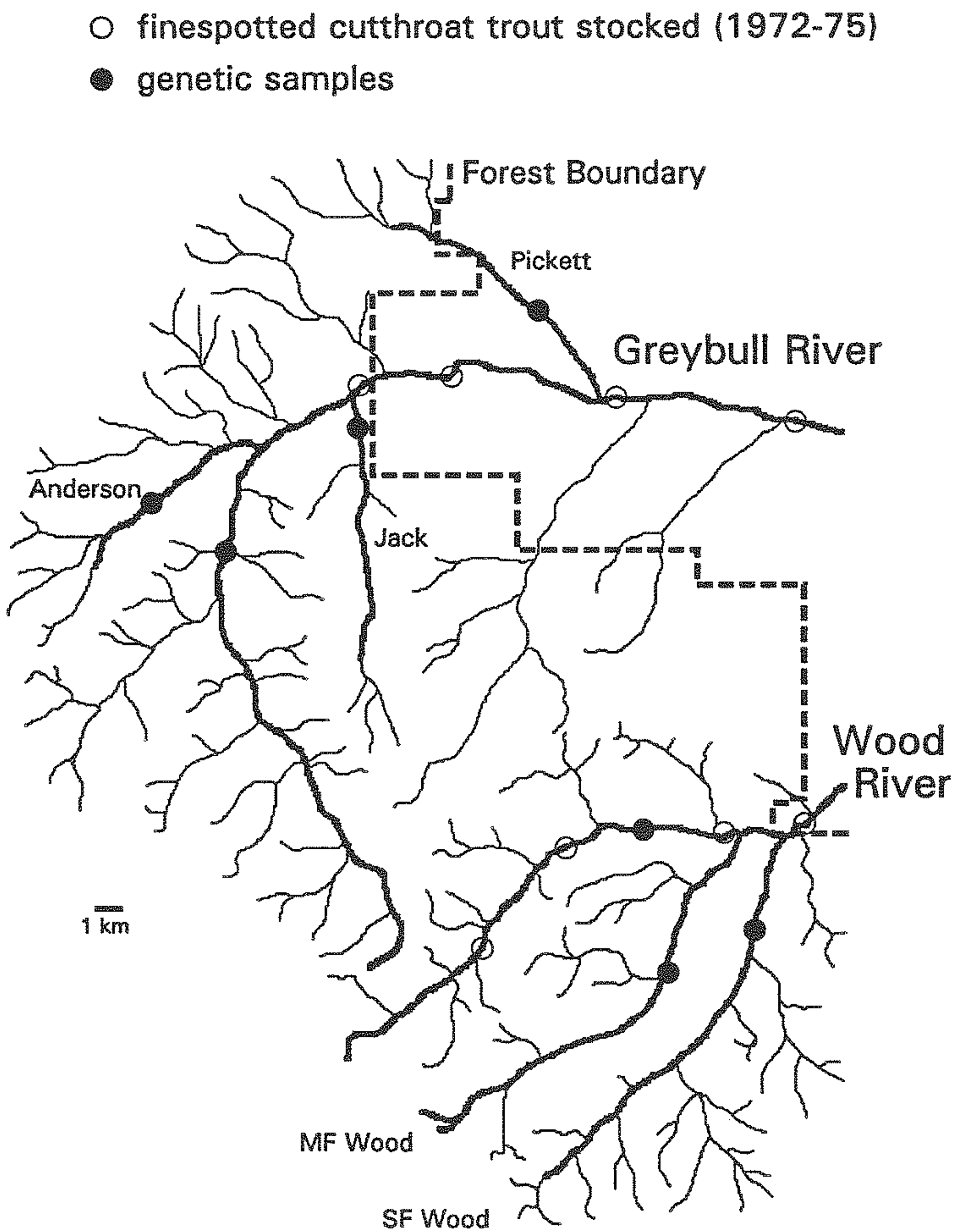
Fifty-six study streams in the Greybull River drainage were sampled with Smith-Root Model 12 battery back-pack electroshockers from June to September 1994. One-pass electrofishing runs (100-m reaches), starting at the confluence of tributaries with the Wood or Greybull river and moving upstream, were performed at approximately 1-km intervals until cutthroat trout were no longer captured in each stream. Reaches immediately below and above fish migration barriers were also sampled.

Captured cutthroat trout were measured (total length in millimeters), weighed (grams) and morphometrically examined for evidence of hybridization with rainbow trout and finespotted cutthroat trout. Criteria for identifying introgression with rainbow trout were: (1) numerous small spots on the head; (2) lack of basibranchial teeth; or (3) white tipped pelvic fins (Behnke 1992). The criteria for identifying hybridization of YSC with finespotted cutthroat trout was spotting patterns intermediate to the characteristic pattern of each subspecies (Behnke 1992).

Genetic Analysis

Cutthroat trout (12-20 fish) were sacrificed from 18 streams throughout the Greybull River drainage (Figure 1). Fish were collected from the midpoint of each stream where

Figure 1. Locations of study streams, genetic samples, and locations where finespotted cutthroat trout were stocked in the drainage.



cutthroat trout were found and above any fish migration barriers (geologic structures at least 1.5 m in height; dry stream reaches, and very high gradient or velocity areas; Stuber et al. 1988). The head, liver, and a sample of muscle tissue were removed from each cutthroat trout, wrapped in aluminum foil, and frozen within 1 hr in liquid nitrogen. The samples from each fish were individually identified as was each fish. The portions of each fish not used for electrophoretic analysis were preserved in 75% ethyl alcohol.

Frozen tissue samples from seven (Greybull River, Anderson Creek, Jack Creek, Pickett Creek, Wood River, South Fork Wood River, and Middle Fork Wood River, Figure 1) of the 18 streams were sent to the WTSGL for genetic analysis. Protein electrophoresis (Allendorf and Phelps 1980; Leary et al. 1984; Leary and Allendorf 1987; Marnell et al. 1987; Perkins et al. 1993) was performed to detect each fish's genetic characteristics at 45 loci coding for proteins present in muscle, liver, or eye tissue (Table 1). Differences in allele frequencies at diagnostic loci were evaluated to determine hybridization with rainbow trout or other cutthroat trout subspecies.

Meristic analysis

Seven meristic features were counted on the preserved cutthroat trout in the laboratory: (1) basibranchial teeth;

Table 1. Enzymes and loci examined in cutthroat trout (E=eye, L=liver, M=muscle).

Enzyme	Loci	Tissue
Adenylate kinase	AK-1*,AK-2*	M
Alcohol dehydrogenase	ADH*	L
Aspartate aminotransferase	sAAT-1*,sAAT-2* sATT-3,4*	L M
Creatine kinase	CK-A1*,CK-A2* CK-B*,CK-C1*,CK-C2*	M E
Dipeptidase	PEPA-1*,PEPA-2*	E
Glucose-6-phosphate isomerase	GPI-A*,GPI-B1*,GPI-B2*	M
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-3*,GAPDH-4*	E
Glycerol-3-phosphate dehydrogenase	G3DHP-1*,G3DHP-2*	L
Isocitrate dehydrogenase	mIDHP-1*,mIDHP-2* sIDHP-1*,sIDHP-2*	M L
L-Iditol dehydrogenase	IDDH*	L
L-Lactate dehydrogenase	LDH-A1*,LDH-A2* LDH-B1*,LDH-B2*,LDH-C*	M E
Malate dehydrogenase	sMDH-A1,2* sMDH-B1,2*	L M
Malic enzyme	mMEP-1*,mMEP-2*,sMEP-1* sMEP-2*	M L
Phosphoglucomutase	PGM-1*,PGM-2*	M
Phosphogluconate dehydrogenase	PGDH*	M
Superoxide dismutase	sSOD-1*	L
Tripeptide aminopeptidase	PEPB*	E
Xanthine dehydrogenase-like	XDHI*	L

(2) gillrakers; (3) pelvic fin rays; (4) scales in the lateral series; (5) scales above lateral line; (6) pyloric caeca; and (7) vertebrae (Behnke 1992). Three independent readers counted each meristic structure on 50 cutthroat trout three different times to assess the repeatability and variation of counts within and among individual readers. Additionally, one reader counted the seven meristic features on 125 additional cutthroat trout to determine mean counts for each structure.

Basibranchial teeth, gill rakers, and pelvic fin rays were counted on the right side of each cutthroat trout. Scales in the lateral series were counted two scale rows above the lateral line starting at the opercle opening continuing to the insertion of the caudal fin. Scales above the lateral line were counted from the lateral line vertically to the anterior of the dorsal fin. Scale counts were done on the right side of each fish. Vertebral counts were completed by dissecting the cutthroat trout and counting the exposed vertebrae. Pyloric caeca were enumerated by stretching the stomach and counting the number of caeca ends. Meristic features were viewed under a dissecting microscope using 30x magnification and reflected light to aid in counting.

Stream elevations (meters) and distance to the location of finespotted cutthroat trout introductions (kilometers) were determined from U.S. Geological Survey 1:24,000 quadrant maps. Wetted stream width was measured at four transects equally spaced through each study reach perpendicular to stream flow, and the mean was computed.

One-way analysis of variance (ANOVA; Krebs 1989) was used to determine possible differences in mean counts of meristic features within and among readers, and between streams. If significant differences were found, Tukey's multiple-comparison test was used to make pairwise comparisons. Simple-linear regression was used to compare elevation, stream size, and distance from finespotted cutthroat trout stocking locations to mean meristic counts from each stream. Statistical analyses were performed using SPSS/PC+ (SPSS Inc. 1991). Significance was determined at $P \leq 0.05$ for all tests.

RESULTS

Genetic analysis

Cutthroat trout were present in 23 of 56 study streams; but no rainbow trout were collected. No cutthroat trout appeared morphometrically to be hybridized with rainbow trout (many small spots on head, white pelvic fin tips, or lack of basibranchial teeth; Behnke 1992). However, cutthroat trout throughout the drainage had spotting patterns indicating that they are hybridized with finespotted cutthroat trout (Figure 2), with the exception of Yellowstone cutthroat trout stocked above fish migration

Figure 2. Spotting patterns of Yellowstone cutthroat trout, finespotted cutthroat trout and a hybrid.

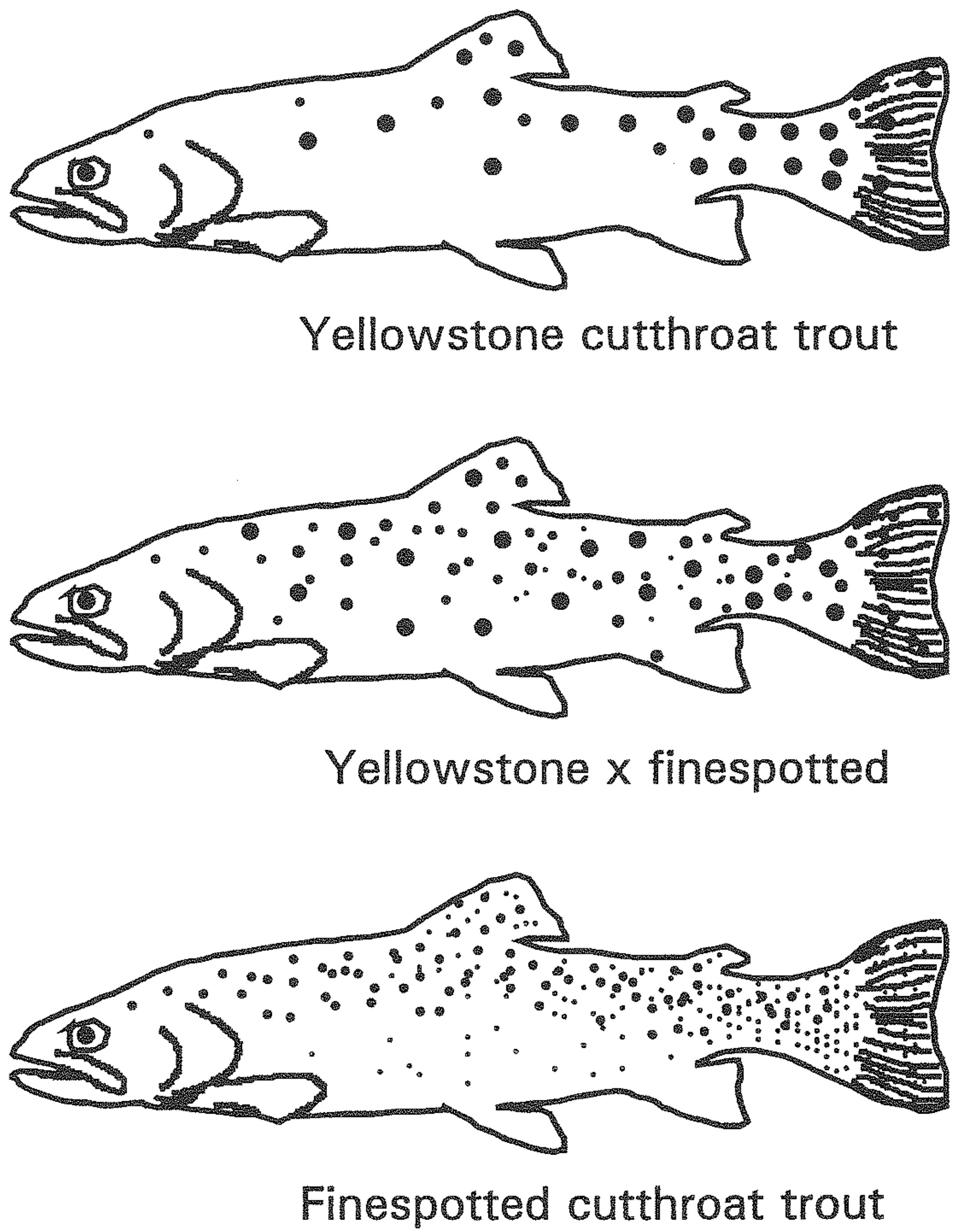


Table 2. Alleles at the loci that distinguish Yellowstone cutthroat trout and rainbow trout.

	Characteristic Alleles	
Locus	Yellowstone	Rainbow
sAAT-1*	165	100,0
CK-A2*	84	100
CK-C1*	38	100,150,38
mIDHP-1*	-75	100
sIDHP-1*	71	100,114,71,40
sMEP-1*	90,100	100
sMEP-2*	110	100,75
PEPA-1*	101	100,115
PEPB*	135	100
PGM-1*	null	100,null

barriers on Cow, Anderson and Eleanor creeks, and the upper Greybull River.

Electrophoretic analysis of fish sampled from the seven streams showed no rainbow trout genes at any of the ten diagnostic gene loci that differentiate YSC and rainbow trout (Figure 3). Finespotted cutthroat trout genes were found in four (Jack Creek (18.4% hybridized, Appendix B), Wood River (5%), South Fork of the Wood River (13.3%), Middle Fork of the Wood River (3.3%)) of the seven streams (Final genetic results, April 14, 1995, WTSGL).

__Meristic analysis__

No significant differences were found among counts by the same reader for any meristic feature (Table 1). All three readers had high agreement among counts for each structure (Table 1). Significant differences among readers were observed for all structures except gillrakers (Table 2). All three readers were significantly different in their counts of pyloric caeca, pelvic fin rays, and scales above the lateral line, while at least one reader was significantly different from the other two readers in counts of vertebrae, basibranchial teeth, and scales in the lateral series (Table 2).

Significant differences in counts of meristic features were observed among fish from the 12 streams (Table 3). No significant relationships or trends were found between mean

Table 3. Mean counts of seven meristic structures of cutthroat trout (n=50). P-values represent the comparison among counts within each reader.

	Count				
Reader	**1**	**2**	**3**	**Structure**	**P**
1	33.0	32.6	32.5	Pyloric caeca	0.90
2	36.9	37.2	36.6		0.95
3	41.2	40.8	41.0		0.99
1	60.4	60.4	60.5	Vertebrae	0.90
2	59.5	59.4	59.6		0.86
3	59.3	59.4	59.3		0.81
1	8.98	8.96	9.02	Pelvic fin rays	0.75
2	8.76	8.84	8.78		0.62
3	9.46	9.38	9.40		0.77
1	18.9	19.0	19.0	Gillrakers	0.93
2	18.8	18.8	18.8		0.98
3	18.3	20.9	18.5		0.42
1	13.8	13.6	13.6	Basibranchial teeth	0.96
2	14.9	15.6	15.4		0.73
3	14.1	14.3	14.2		0.98
1	177.8	178.5	177.8	Scales in lateral series	0.96
2	188.2	186.8	187.5		0.88
3	187.4	187.2	187.6		0.99
1	43.9	44.0	44.1	Scales above lateral line	0.98
2	56.3	56.0	56.9		0.71
3	42.5	42.6	42.3		0.89

Table 4. Mean meristic counts of seven meristic features of cutthroat trout among three independent readers. Means not different significantly indicated by underline.

	Reader			
Structure	**1**	**2**	**3**	**P**
Pyloric caeca	32.7	36.9	41.0	< 0.0001
Vertebrae	60.5	59.5	59.3	< 0.0001
Pelvic fin rays	9.0	8.8	9.4	< 0.0001
Gillrakers	18.9	18.8	19.3	0.83
Basibranchial teeth	13.7	15.3	14.2	0.0026
Scales in lateral series	178.0	187.5	187.4	< 0.0001
Scales above lateral line	44	56.4	42.5	< 0.0001

Table 5. Mean meristic counts for the twelve study streams with more than five cutthroat trout. Means not significantly different (Tukey, $P \leq 0.05$) are indicated by the same superscript. Significant correlation coefficients ($P \leq 0.05$) are indicated in bold.

Location	Pyloric caeca	Vertebra	Pelvic fin ray	Gill-rakers	Basi-branchial teeth	Scales lateral line	Scales above lateral line
Chimney	36.9[b,d]	58.2[a]	9.3[a,c]	19.9[a]	11.7[a]	175.5[a]	39.4[a]
Cow	51.3[a]	58.5[a]	9.4[b,c]	18.4[a,b]	13.5[a]	178.1[a]	37.1[a]
Anderson	51.4[a]	59.0[a,b]	9.6[b,c]	18.4[a,b]	13.0[a]	181.6[a]	40.1[a]
Jack	38.5[a,b,d]	58.0[a]	9.1[a,c]	19.9[a,b]	15.9[a,b]	177.9[a]	39.9[a]
Brown	49.1[a]	58.8[a]	9.1[a]	18.5[a,b]	11.4[a]	177.6[a]	41.6[a]
Greybull[1]	45.7[a,b]	57.9[a]	9.1[a]	19.4[a,b]	12.2[a]	180.6[a]	38.8[a]
Venus	39.7[a,b,d]	59.0[a,b]	9.2[a,c]	19.2[a,b]	15.7[a,b]	192.1[a,b]	41.4[a,b]
Warhouse	40.6[a,b,d]	58.1[a]	9.0[a]	19.2[a,b]	14.1[a]	182.6[a]	38.8[a]
Deer	36.9[b,d]	59.1[a]	9.3[b,c]	18.2[b]	16.1[a,b]	187.2[a]	41.4[a]
Middle Fork Wood River	44.6[a,b]	58.5[a]	9.1[a,c]	19.4[a,b]	13.2[a]	183.1[a]	39.8[a]
South Fork Wood River	28.9[d]	60.6[b]	9.9[b,c]	17.8[b]	14.0[a,b]	207.3[b]	45.5[b]
Greybull[2]	37.8[a,b,d]	58.0[a]	9.7[b,c]	18.7[a,b]	21.8[b]	189.5[a,b]	39.0[a]

Table 3. Continued.

Location	Pyloric caeca	Vertebra	Pelvic fin ray	Gill-rakers	Basi-branchial teeth	Scales lateral line	Scales above lateral line
Mean	**42.3**	**58.8**	**9.2**	**18.8**	**14.0**	**182.7**	**40.4**
Elevation	0.34	-0.31	0.25	-0.07	0.40	-0.10	-0.58
Stream width	0.15	-0.17	-0.08	0.24	-0.21	0.03	-0.16
Distance from finespotted stocking sites	0.23	-0.24	0.33	-0.11	0.40	-0.01	-0.51

counts of meristic features in each stream and stream elevation, size, or the distance to locations where finespotted cutthroat trout were stocked (Table 3). Means for each of the seven meristic features were: (1) pyloric caeca, 42.3; (2) vertebrae, 58.8; (3) pelvic fin rays, 9.2; (4) gillrakers, 18.8; (5) basibranchial teeth, 14; (6) scales in the lateral series, 182.7; and (7) scales above the lateral line, 40.4.

DISCUSSION

Stocking history

Trout stocking has occurred in the Greybull River system since 1915 (Lenihan 1915, 1916; Yekel 1980). Records of the WGFD indicate that 13 streams within the study area have been stocked (Appendix A).

Rainbow trout were stocked in four streams, Greybull River (1915), Wood River (1915), Timber Creek (1936, 1955) and Pickett Creek (1949), within the study area, as well as another Greybull River tributary, Meeteetsee Creek (1946). However, rainbow trout have not been collected within the study area since shortly after these plantings.

Brook trout were introduced into the study area in 1916 in the Greybull River and between 1935 and 1949 in Francs Fork, Pickett Creek, Timber Creek, Blanchette Creek, and JoJo Creek. Presently they are found in Deer Creek and the Wood River.

Cutthroat trout have been stocked regularly from 1936 to the present. The subspecies of cutthroat trout used in many introductions prior to 1970 were not identified and were simply referred to as blackspotted trout or cutthroat trout. These fish are presumed to have been progeny of fish that are now known as YSC from Yellowstone Lake or finespotted cutthroat trout from the Snake River drainage. From 1972 to 1975, finespotted cutthroat trout were stocked in the Greybull and Wood rivers. More recently, YSC have been stocked into the upper Greybull River, Cow Creek, Anderson Creek, Eleanor Creek, and Venus Creek (1988 and 1993). These stocked fish currently inhabit nearly 20 km (12.5%) of occupied stream length within the Greybull River drainage (Chapter 2).

Morphometric analysis

Due to past stocking practices and the ability of cutthroat trout to migrate throughout the drainage (Heggenes et al. 1991), the potential exists for the native YSC present in the drainage to no longer be genetically pure. The cutthroat trout on which I examined morphometric features showed no indication of hybridization with rainbow trout; however, integration of spotting patterns between Yellowstone and finespotted forms (Behnke 1992) were seen throughout the drainage, except in those streams recently stocked, upstream from barriers with pure YSC in 1988 and

1993. However, distinction between YSC and finespotted cutthroat trout is difficult due to natural variations in spotting patterns (Leary et al. 1985; Behnke 1992); thus, this is not a definitive method to determine hybridization.

<u>Electrophoretic analysis</u>

Electrophoresis indicated that rainbow trout have not hybridized with cutthroat trout in the Greybull River drainage. Because protein analysis is a reliable method of assessing hybridization between cutthroat trout and rainbow trout (Leary et al. 1987; Carmichael 1993) it is probable that introduced rainbow trout did not hybridize with native cutthroat trout. Therefore, rainbow trout have not been a factor contributing to the decline of YSC within the Greybull River drainage.

Finespotted cutthroat trout genes were found, using electrophoretic analysis, in fish from four of the seven streams sampled (Final genetic results, April 14, 1995, WTSGL). Jack Creek, the South and Middle forks of the Wood River, and the Wood River, contained some level of introgression between YSC and finespotted cutthroat trout. Finespotted cutthroat trout were stocked in the Greybull River at the mouth of Jack Creek and in the Wood River near the confluence of both the South and Middle Forks of the Wood River from 1972 to 1975 (Yekel 1980). Thus, it would be expected that these sites would have the highest levels of introgression with finespotted cutthroat trout. The Greybull River did not show electrophoretic evidence of hybridization; however, lower levels of introgression (< 7.5%) may not be detectable (Final genetic results, April 14, 1995, WTSGL). Therefore, finespotted genes may be present throughout the drainage, but are undetectable.

There is both morphometric and electrophoretic evidence that YSC and finespotted cutthroat trout hybrids are present in the Greybull River drainage. These trout can migrate freely throughout most of the drainage downstream from barriers (Heggenes et al. 1991); consequently, I conclude that pure YSC do not currently occur in the Greybull River drainage, except in four streams upstream from barriers where fish from hatchery stocks were planted in 1988 and 1993.

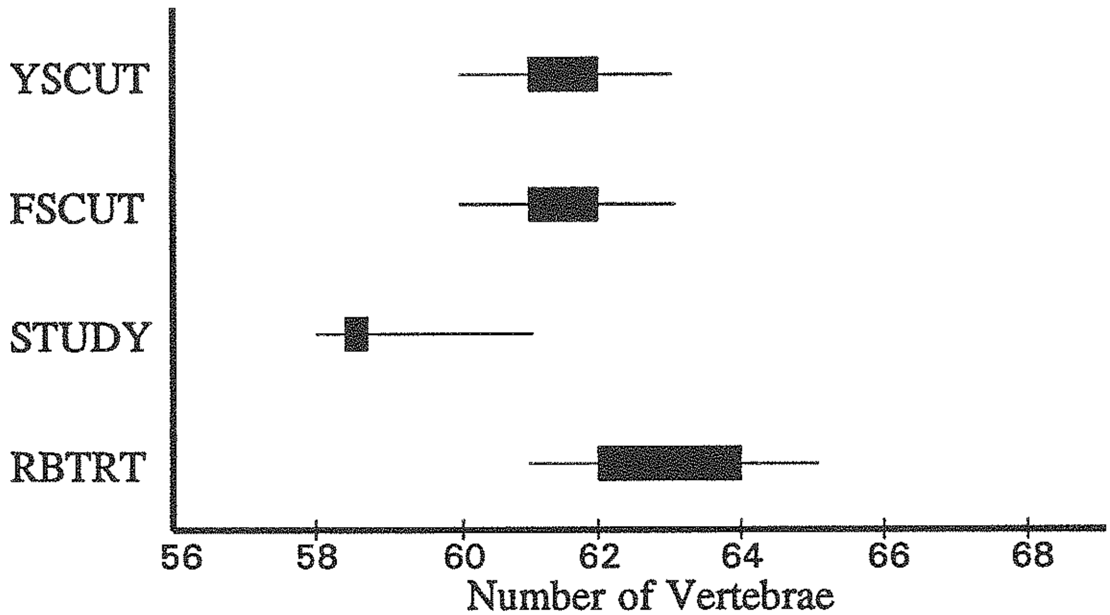
If it is assumed that YSC and finespotted cutthroat trout are separate subspecies (Behnke 1992) and that the past distribution of YSC within the drainage was similar to the distribution of cutthroat trout today (45% of the 355 km of permanent stream waters, Chapter 2), then genetically pure, wild YSC probably no longer remain within the Greybull River drainage. Genetically pure YSC occupy approximately 5% of the drainage area; however, these populations are hatchery supported and cannot be classified as "wild." In contrast, if YSC and finespotted cutthroat trout are not separate subspecies, but rather phenotypic variations of the same species, genetically pure, wild YSC still occupy 40% of the drainage, while genetically pure, hatchery YSC make up the remaining 5%.

<u>Meristic counts</u>

Due to the relatively simple methodology, meristic counts have been used to assess hybridization in trout. Marnell et al. (1987) found close agreement between meristic and electrophoretic results with YSC and westslope (*O. c. lewisi*) cutthroat trout, two subspecies with a relatively large evolutionary separation (Shiozawa and Williams 1988; Behnke 1992). However, Loudenslager and Gall (1980) and Loudenslager and Kitchen (1979) were unable to find consistent differences between more closely related subspecies (YSC and finespotted cutthroat trout). Recent research has shown that meristic comparisons can provide potentially misleading information (Busack and Gall 1981; Leary et al. 1984, 1985) and Behnke (1992) warns that meristic counts and morphological descriptions are often specific to only localized populations and can differ among populations of the same subspecies due to regional differences in meristic characteristics.

Behnke (1992) described YSC as having 60-63 vertebrae (typically 61-62), 150-200 scales in the lateral series (165-180), 25-50 pyloric caeca (35-43), 17-23 gillrakers (19-20), and 9-10 pelvic fin rays (9; Figure 3).

Figure 3. Meristic comparisons of pyloric caeca, vertebrae, basibranchial teeth, and scales in the lateral series for Yellowstone cutthroat trout (YSCUT), finespotted cutthroat trout (FSCUT), rainbow trout (RBTRT) from Behnke (1992), and the current study (STUDY). Lines indicate ranges of counts and bars indicate typical counts for that species or subspecies.



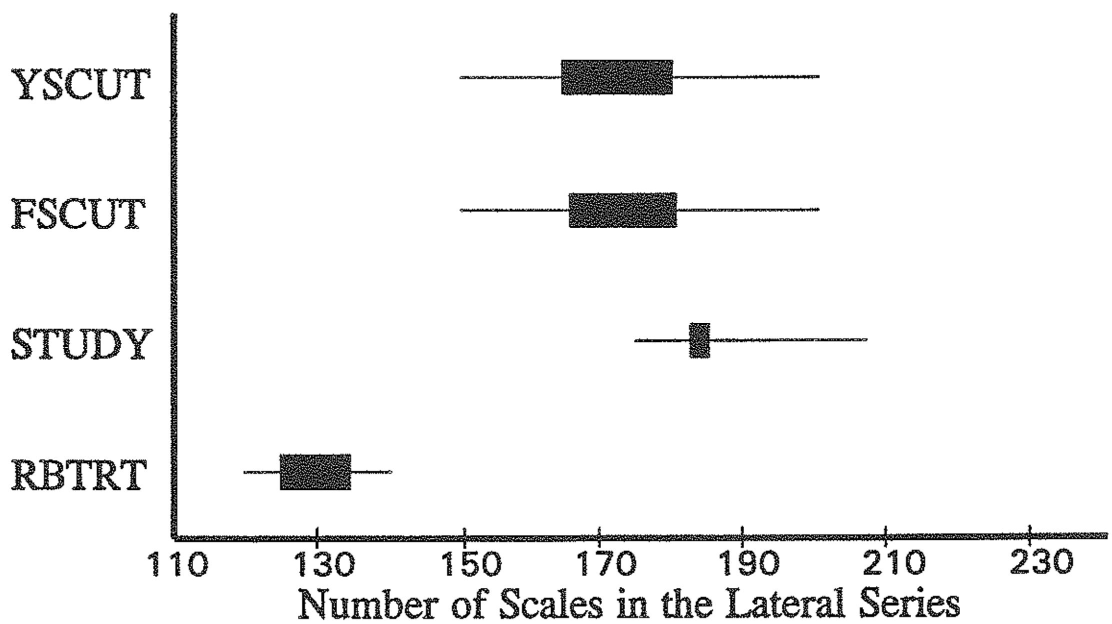
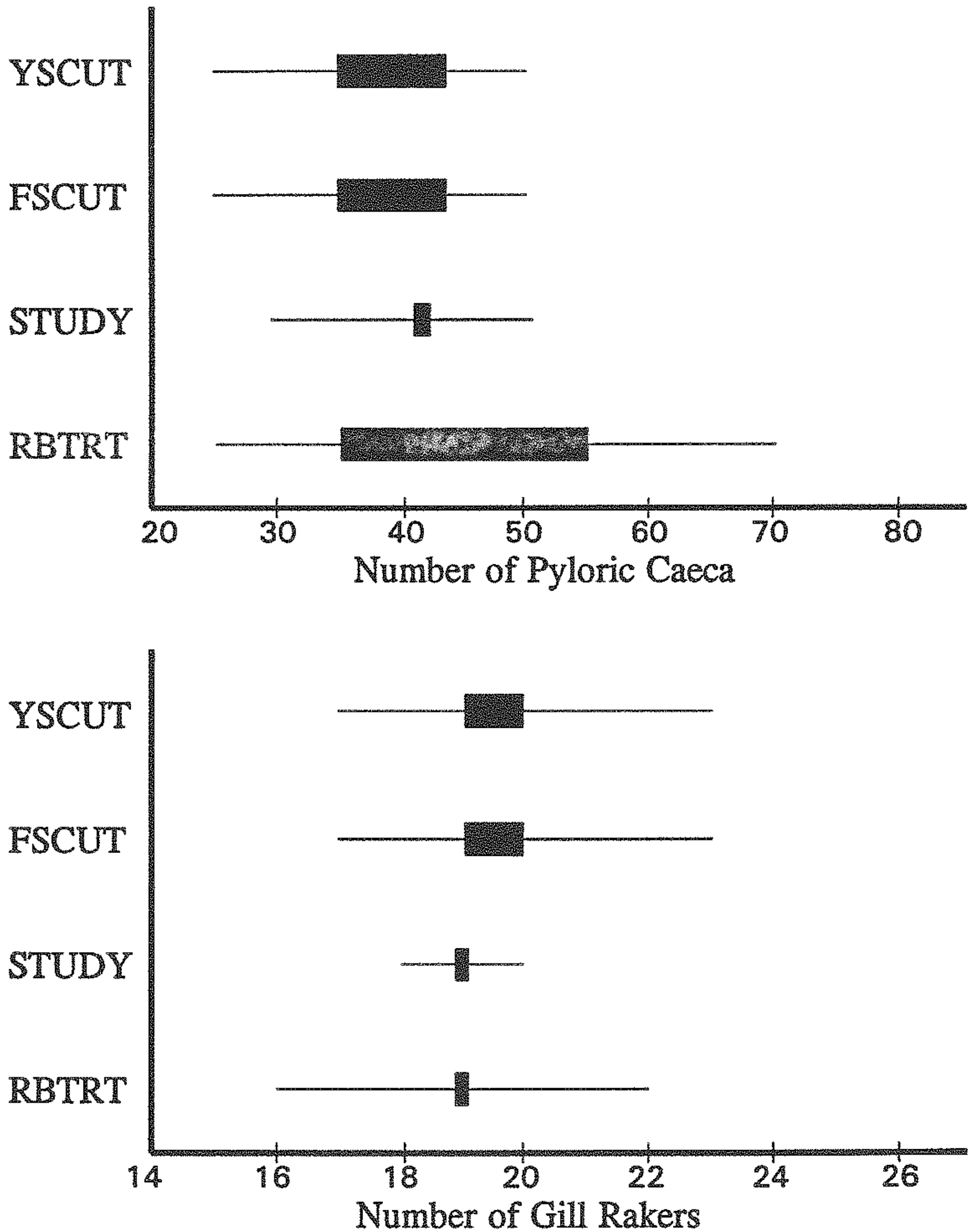


Figure 3. Continued.



Finespotted cutthroat trout have counts nearly identical to those of the YSC, whereas rainbow trout have fewer scales in the lateral series (120-140), but generally, more pyloric caeca (25-70) and vertebra (61-65). Mean counts of meristic features of cutthroat trout in the Greybull River drainage were similar to those of YSC (Behnke 1992), corroborating electrophoretic evidence that there is no evidence of rainbow trout hybridization. However, meristic counts provided little insight in differentiating YSC and finespotted cutthroat trout.

Meristic counts differed significantly among sites. Behnke (1992) suggested that local influences may account for differences between sites; however, no trends or significant relationships were found between counts and elevation, stream size, or distance from locations where finespotted cutthroat trout were stocked. Although, in no case were the counts significantly different from the ranges described by Behnke (1992). Other environmental influences, such as temperature and latitude (Barlow 1961), or reader variation, may account for the differences among sites.

Because meristic counts continue to be utilized to assess the genetic status of cutthroat trout populations (Binns 1977; Remmick 1981; Hadley 1984; Behnke 1992), even though they have been shown to be inaccurate in many situations (Leary et al. 1983, 1984, 1985), I felt it was important to determine the amount of variation and repeatability in meristic counts among different readers. No significant differences were found in the repeatability of meristic counts by individual readers suggesting that there is little variation among successive counts on the same structure by one reader; therefore, one count of each structure is probably adequate. However, significant differences in mean meristic counts were apparent among readers. It was difficult for separate readers to enumerate the meristic features similarly, suggesting that meristic studies performed by separate researchers may provide inconsistent results. Therefore, it is difficult to compare meristic counts among readers or studies. However, although readers differed significantly in mean count values, no means were outside the limits suggested by Behnke (1992).

CONCLUSIONS

(1) Rainbow trout are not present in the Greybull River drainage and there appears to be no hybridization between cutthroat trout and rainbow trout in the drainage;

(2) Native Yellowstone cutthroat trout in the drainage appear to have hybridized with introduced finespotted cutthroat trout. No cutthroat trout except those introduced by the WGFD above fish migration barriers should be considered genetically pure Yellowstone cutthroat trout;

(3) Morphological comparisons corroborate the electrophoretic evidence of hybridization of Yellowstone and

finespotted cutthroat trout in the Greybull River drainage.

(4) Meristic counts varied significantly among readers; therefore, comparisons among studies and readers are likely to be confounded by reader error;

(5) If finespotted cutthroat trout are considered a distinct subspecies, there probably are no genetically pure, wild Yellowstone cutthroat trout in the Greybull River drainage. However, if finespotted cutthroat trout are considered to be a form of Yellowstone cutthroat trout and not a separate subspecies, Yellowstone cutthroat trout are present throughout the Greybull River drainage.

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CHAPTER IV: FACTORS INFLUENCING ABUNDANCE OF CUTTHROAT TROUT

INTRODUCTION

Identification of important components of stream habitat is essential in understanding environmental and biological influences on the abundance of cutthroat trout (*Oncorhynchus clarki*) in high-elevation systems. The variability in density of cutthroat trout among mountain streams is believed to be related to the quality of physical habitat (Moore and Gregory 1989). Stream habitat quality is a function of the hydrology, geology and riparian features of the watershed (Bjornn et al. 1991).

Trout growing in small mountain streams tend to select certain types of habitat (Bisson et al. 1988). It is assumed that if these habitat types are of high quality they will support a larger standing stock of trout; therefore, standing stock is an index of habitat quality (Binns and Eiserman 1979).

The concept of multivariate habitat control of fish density has been described (Platts 1974; Binns and Eiserman 1979). Numerous physical, biological and chemical factors may interact to affect cutthroat trout densities in stream systems. Many studies have investigated the influence of habitat on trout standing stocks (Minshall et al. 1983; Scarnecchia and Bergersen 1986; Kozel and Hubert 1989; Woodward et al. 1989; Bozek and Hubert 1992); however, biotic and abiotic factors that govern standing stocks of cutthroat trout in high-elevation mountain streams are not well documented.

The Greybull River drainage is volcanic in origin with a steep and rugged landscape (Keefer 1972). Past geologic history, along with present rock deposits and topography, combine to make the Greybull River drainage geologically unstable. The mountain streams have steep longitudinal profiles and low biotic productivity (Hansen and Grover 1973). Stream substrates and banks are predominately erosive volcanic sediments and rubble (Hansen and Grover 1973; Zafft and Annear 1992). High spring flows in combination with steep gradients cause extensive streambank and bed erosion resulting in channels that shift regularly (Kent 1984; Zafft and Annear 1992), are strewn with large, angular rock (boulder and rubble; Hanzel 1959), are poorly defined, and provide limited fish habitat (Moore and Gregory 1989). Most previous work on trout standing stocks in high-elevation systems has been done in relatively stable drainages (Kozel and Hubert 1986; Kozel et al. 1990; Bozek and Hubert 1992); thus, this study is unique in that it evaluates cutthroat trout standing stocks in relation to habitat in a high-gradient, unstable, high-elevation system. My objective was to identify physical habitat features that influence the density of cutthroat trout in high-elevation (> 2000 m) streams in the Greybull River drainage.

METHODS

Sampling was conducted from June to September 1994 on 21 streams within the Greybull River drainage that contained cutthroat trout populations. A 100-m reach was sampled on each stream near the midpoint of the length of stream occupied by cutthroat trout.

Stream reaches were isolated by placing 1.25-cm-mesh block nets at the upstream and downstream ends. Three electrofishing passes were performed using Smith-Root Model 12 POW battery-powered back-pack shockers and fish were collected and counted after each pass. Captured cutthroat trout were weighed (grams) and total length (millimeters) was measured. Population estimates and 95% confidence intervals were calculated using the maximum likelihood estimator method of estimating fish populations from successive removals (Zippin 1956; Riley and Fausch 1992) with the program CAPTURE, model Mb(h) (White et al. 1982).

Within each study reach, 28 physical, chemical and biological habitat variables were measured or calculated. Stream reach location and elevation (meters) were determined from 1:24,000 U.S. Geological Survey topographic maps. Stream gradient was estimated with a clinometer over the 100-m length. Mean wetted width was measured perpendicular to stream flow at four transects spaced equally through each study reach. Thalweg depth (meters) and bankfull channel width (meters) were also measured at each transect (Fausch and Northcote 1992).

An index of spawning habitat, the number of sites in each reach with sizes of gravel preferred for spawning by cutthroat trout (0.6-7.6 cm; Cope 1957), was calculated to give an estimate of available spawning habitat. Potential spawning sites, irregardless of size or location in the stream, were enumerated and reported as number of sites per kilometer. The proportion of pool, riffle, and run habitat (defined by Bisson et al. 1982) in the stream reach was measured.

Substrate composition was visually estimated across three equally-spaced transects (not the same transects used for width and depth estimates) and classified by proportion into four categories: (1) boulder (> 30.5 cm in diameter); (2) rubble (7.6-30.5 cm); (3) gravel (0.25-7.6 cm); and (4) sand/silt (< 0.25 cm, Rahel and Hubert 1991; WGFD stream survey protocol).

Stream channel stability was determined using the R-1 Stream Reach Inventory and Channel Stability Evaluation developed by Pfankuch (1975). Ratings were made of 14 stability indicators ranging from landform slope to aquatic vegetation, summed, and scores were placed into one of four categories: (1) excellent = < 34; (2) good = 34-68; (3) fair = 69-102; and (4) poor = > 103 points. The length of eroding stream banks (meters) was measured on both sides of the 100-m reach and identified by the presence of bare or cut banks.

Alkalinity (milligrams $CaCO_3$/liter), hardness (milligrams/liter), and acidity (pH) were estimated with Hach Chemical Water Quality tests. A total dissolved solid meter was used to measure dissolved solids, and temperatures (degrees Celsius) were taken.

Water velocity was estimated using the float method (Gordon et al. 1992) and corrected by a factor of 0.85. Floats were timed (four replicates) over three transects located on a straight section of stream $\geq$ 5 m in length. Wetted widths and depths were measured across each transect to allow calculation of stream volume (cubic meters), discharge (cubic meters second), and water surface area (square meters). Wetted width to depth ratios (Lanka et al. 1987; Kozel and Hubert 1989; Fausch and Northcote 1992) and wetted width to bankfull-channel width ratios were calculated.

Trout cover, identified as undercut banks, surface turbulence, aquatic and overhanging terrestrial vegetation, large woody debris, large rocks, and other submerged debris (Banks et al. 1974; Binns and Eiserman 1979) was measured and calculated as a proportion of total stream reach area.

Pearson correlation coefficients and simple-linear-regression analysis were used to determine relationships between measured standing stocks and the habitat variables

(Krebs 1989). Analyses were performed using SPSS/PC+ (SPSS Inc. 1991). Significance was determined at $P \leq 0.05$ for all tests.

RESULTS

Cutthroat trout population estimates and standing stocks (kilograms/hectare) for 21 study reaches are presented (Table 1). Populations ranged from 1 fish/100 m in the upper section of the Greybull River to 57 fish/100 m in the Wood River, while standing stocks varied from 5 kg/ha on the upper Greybull River and Red Creek to 92 kg/ha in West Timber Creek.

The mean, range, and standard deviation for each of the 28 habitat variables measured were computed (Table 2). Correlation coefficients between each variable and standing stocks are given (Table 3). Only cover ($P = 0.001$, Figure 1) and bankfull width ($P = 0.040$, Figure 2) were significantly correlated with cutthroat trout standing stocks. Riffle habitat and bankfull width were significantly correlated with cover ($P = 0.003$ and 0.041, respectively, Table 4), suggesting they are not independent in predicting trout standing stocks. Cover and elevation or gradient were not related (Table 4).

Table 1. Estimates of population size and standing stock (kilograms/hectare) for cutthroat trout in 21 study reaches (100 m) in the Greybull River drainage. Values presented in parenthesis are 95% confidence intervals.

Stream	Population estimate	Standing stock
Anderson Creek	9 (9-9)	32
Brown Creek	20 (20-20)	37
Chimney Creek	14 (14-14)	58
Cow Creek	12 (10-31)	57
Deer Creek	3 (3-3)	21
Dundee Creek	2 (2-2)	9
Eleanor Creek	18 (18-18)	57
Francs Fork	20 (20-27)	65
Greybull River (upper)	1 (1-5)	5
Greybull River (lower)	40 (40-51)	35
Jack Creek	23 (23-24)	60
Mabel Creek	1 (1-1)	18
Middle Fork Wood	14 (14-14)	52
Pickett Creek	9 (9-9)	21
Piney Creek	5 (5-5)	50
Red Creek	1 (1-1)	5
South Fork Wood	41 (41-45)	70
Venus Creek	7 (7-7)	33
Warhouse Creek	15 (15-18)	89
West Timber Creek	24 (24-24)	92
Wood River	57 (55-66)	69

Table 2. Mean, range, and standard deviation for 28 habitat variables measured in the Greybull River drainage.

Variable	Mean	Range	SD
Elevation (m)	2459	2120-2836	209
Gradient (%)	4.24	1.0-11.0	2.9
Width (m)	4.55	1.36-15.76	3.35
Depth (m)	0.26	0.09-0.49	0.11
Bankfull width (m)	22.9	5.0-50.0	13.7
Spawn rating (#/km)	40.0	0-270.0	55.9
Pool habitat (%)	20.1	0-35	11.0
Run habitat (%)	25.5	0-50	17.9
Riffle habitat (%)	53.3	25-100	20.3
Boulder substrate (%)	22.9	0-60	16.4
Rubble substrate (%)	45.0	15-70	13.4
Gravel substrate (%)	24.5	5-80	16.0
Sand/silt substrate (%)	6.4	0-15	5.5
Channel stability	83.0	59-105	16.2
Eroding banks (m)	87.1	10-200	60.6
Alkalinity (mg $CaCO_3$/L)	69.5	34.2-102.6	20.7
Hardness (mg/L)	59.4	34.2-102.6	22.7
TDS	32.7	0-72	22.7
Temperature (C)	13.8	10-22.8	3.1
Acidity (pH)	7.82	7.5-8.25	0.25
Velocity (m/s)	0.60	0.35-1.0	0.17
Discharge (cms)	0.46	0.03-1.74	0.46

Table 2. Continued.

Variable	**Mean**	**Range**	**SD**
Area (m^2)	457.1	136-1576	336.0
Volume (m^3)	142.2	13.4-634.4	161.2
Wetted Width/ Depth Ratio	17.3	4.1-39.2	7.7
Wetted Width/ Bankfull Width Ratio	0.23	0.08-0.44	0.11
Standing stock (kg/ha)	44.6	5-92	25.7
Cover (%)	11.4	1-26	8.6

Table 3. Correlation coefficients for habitat variables in relation to standing stocks of cutthroat trout. Significant relationships indicated in bold* ($P \leq 0.05$).

Habitat Variable	r
Elevation	-0.33
Gradient	-0.33
Mean width	0.12
Mean depth	0.10
Mean bankfull width	**0.45***
Spawning habitat rating	-0.06
Pool habitat	0.14
Run habitat	0.27
Riffle habitat	-0.39
Boulder substrate	-0.04
Rubble substrate	-0.17
Gravel substrate	-0.31
Sand/Silt substrate	-0.33
Channel stability	-0.25
Eroding stream banks	0.30
Alkalinity	-0.10
Hardness	-0.10
TDS	0.10
Temperature	0.07
Acidity	-0.05
Velocity	-0.04
Discharge	0.09

Table 3. Continued.

Variable	r
Area	0.12
Volume	0.11
Width/Depth Ratio	0.02
Width/Bankfull Ratio	-0.36
Cover	**0.68***

Table 4. Correlation matrix for habitat variables closely related to standing stocks. Significant relationships indicated in bold* ($P \leq 0.05$).

	Gradient	Riffle	Cover Width	Bankfull
Elevation	0.223	0.072	-0.188	0.010
Gradient		-0.234	-0.208	**-0.567***
Riffle			**-0.612***	0.064
Cover				**0.450***

Figure 1. Linear relationship between cutthroat trout standing stocks and cover.

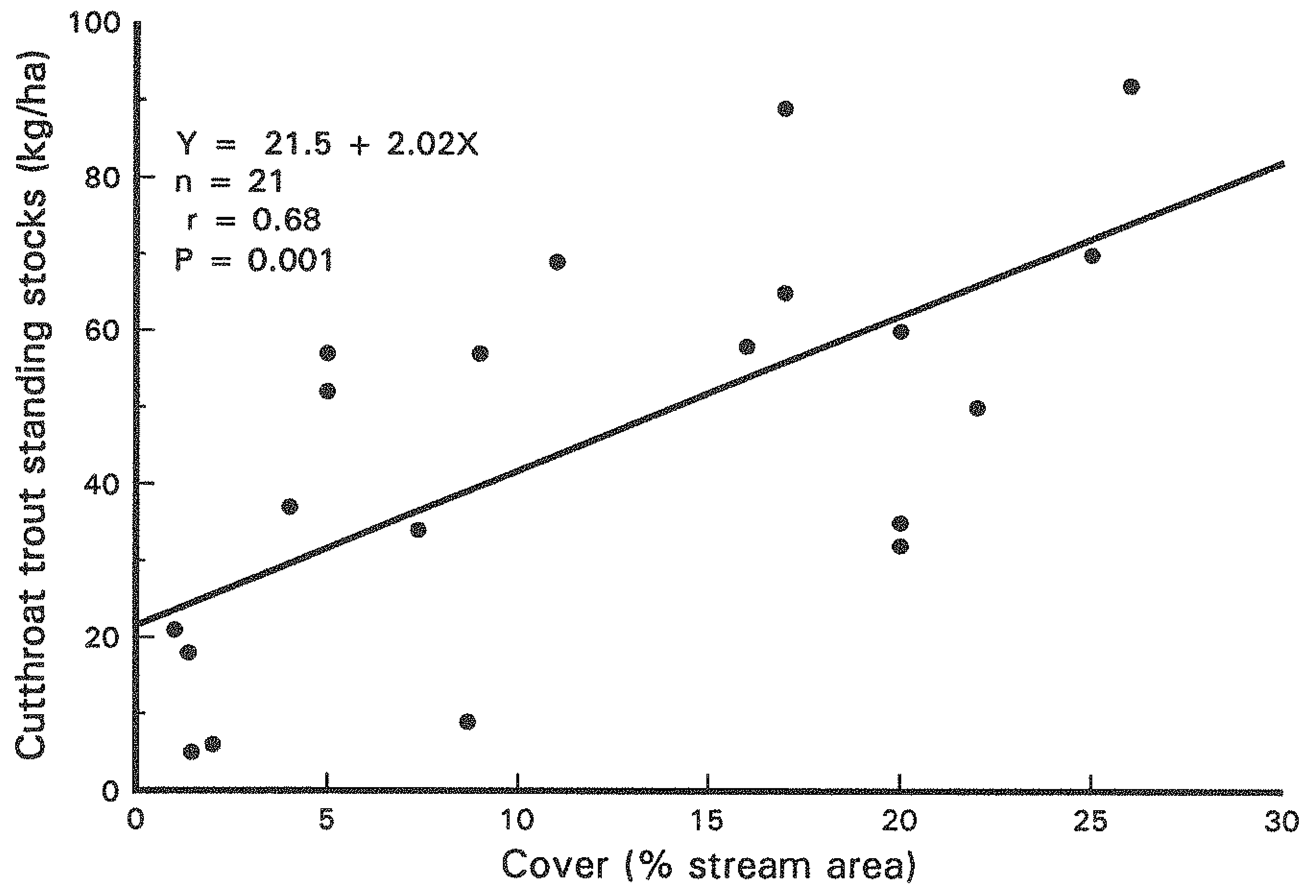
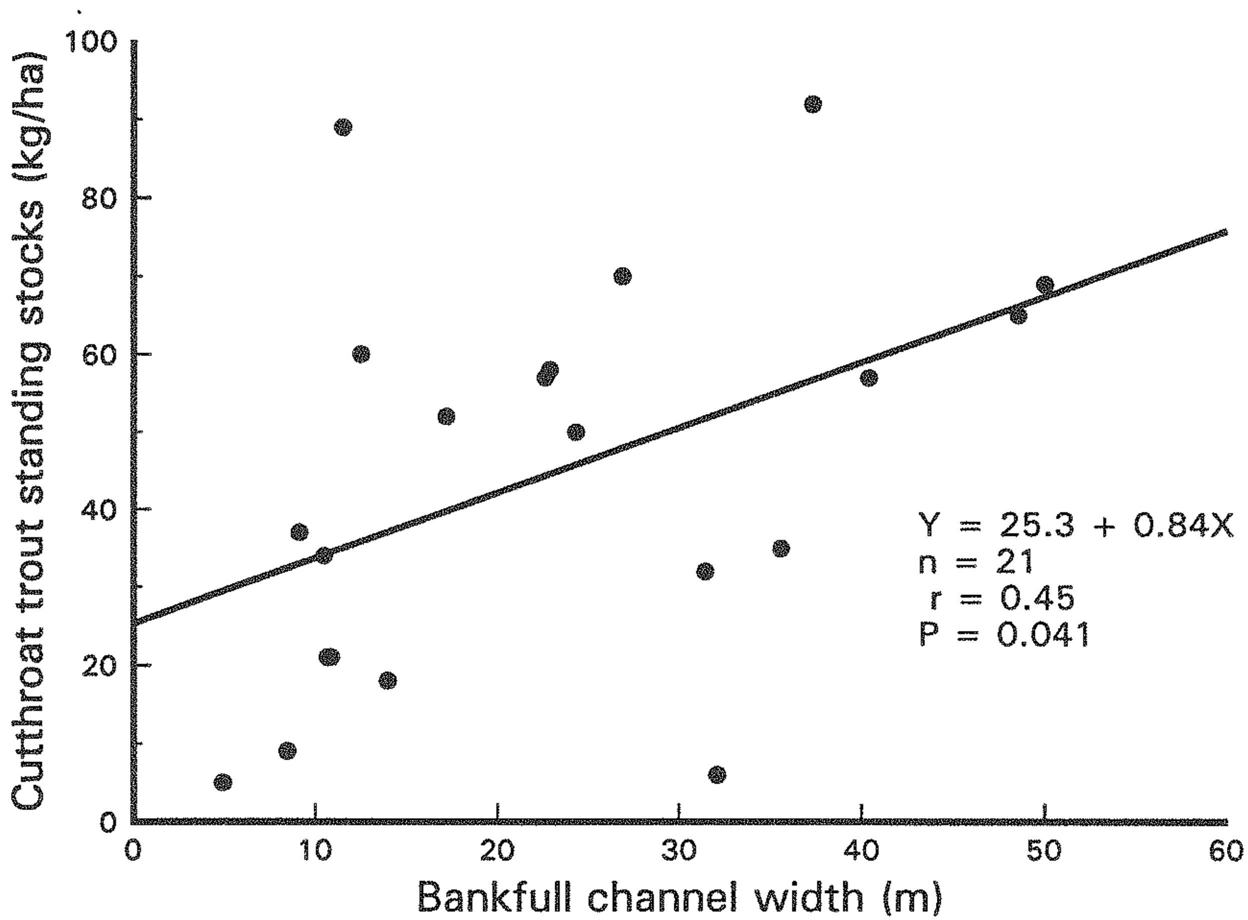


Figure 2. Linear relationship between cutthroat trout standing stocks and bankfull channel width.



DISCUSSION

Cutthroat trout abundance in the 21 high-elevation streams in the Greybull River drainage tended to be lower than standing stocks of salmonids throughout Wyoming. Wiley (1992) reported that 80% of measured trout stocks in Wyoming streams are $\leq$ 134.5 kg/hectare, while 55% are $\leq$ 67.3 kg/hectare. In the Greybull River system, 100% and 77%, respectively, of the streams were in the two categories.

Similar to the Greybull River drainage, Varley and Gresswell (1988) reported wide variability (range 7-145 kg/ha, mean 48 kg/ha) in cutthroat trout standing stocks in 23 mountain streams in Wyoming and Yellowstone National Park. In the Greybull River drainage, standing stocks were similar (5-92 kg/ha with a mean of 45 kg/ha).

The streams in the Greybull River drainage have large fluctuations in flow, are unstable, steep, and contain limited habitat (Chapter 2); thus, it would be expected that environmental conditions would limit cutthroat trout standing stocks. However, standing stock estimates from the Greybull River, although lower than other high-elevation streams in the Medicine Bow Mountains of Wyoming (Kozel and Hubert 1986; Kozel et al. 1990), are similar to other streams in Wyoming and the West (Wiley 1992).

Because habitat and quality are closely tied to cutthroat trout abundance, it is important to determine the habitat factors influencing cutthroat trout abundance (Moore and Gregory 1989). Because the study sites were selected to sample fish for genetic analysis, a sample representing the overall diversity of habitat was not obtained.

The amount of cover and the bankfull channel width were the only variables significantly related to cutthroat trout standing stocks. Cover, defined as sheltered stream areas where trout can rest or hide from predators (Arnette 1976; Griffith and Smith 1993), has been shown to be a critical factor when evaluating standing stocks. Water depth, large rocks and other submerged obstructions, undercut banks, aquatic and overhanging vegetation, and large woody debris have all been identified as cover (Binns and Eiserman 1979); however, stream habitat within the study area was predominately boulder pools. Wilzbach (1985), Cunjak and Power (1987), Kozel and Hubert (1989), Bjornn et al. (1991) and Fausch and Northcote (1992) have all shown that cover is positively correlated with trout biomass. Kozel and Hubert (1989) and Kozel et al. (1990) reported that various components of cover explained the majority of variation in brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) standing stocks in high mountain streams. Within the Greybull River system, cover, in the form of boulder pools, was the main influence on variation in cutthroat trout standing stocks. Pools formed by boulders appear to be the most important factor affecting cutthroat trout standing stocks in the Greybull River drainage.

Bankfull channel width was also related to fish biomass; however, it was also correlated with cover. Because bankfull channel width was related to cover, it was not independently related to fish biomass. It is likely that bankfull channel width is a measure of stream size, and that more pools are present in larger streams at lower elevations and with lower channel slopes (Kennedy and Strange 1982; Chisholm and Hubert 1986; Scarnecchia and Bergersen 1986; Kozel and Hubert 1989; Bozek and Hubert 1992).

CONCLUSIONS

(1) Cutthroat trout standing stocks (range 5-92 kg/ha, mean 48 kg/ha) within streams in the Greybull River drainage were lower than what is seen through much of Wyoming, but similar to other streams in Yellowstone National Park and northwestern Wyoming; and,

(2) Cover and bankfull channel width were related to trout standing stocks within the system. Cover, predominately in the form of boulder pools, appears to be the most important habitat variable influencing cutthroat trout standing stocks.

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CHAPTER V: AGE AND GROWTH

INTRODUCTION

Various fish population statistics, including age distribution and growth, are typically estimated by fishery managers to monitor and manage trout populations. Age and growth estimates are often compared to detect or diagnose environmental changes or ecological conditions influencing growth (Hammers and Miranda 1991). Estimating age structure, year-class strength, and mortality, or calculating indices such as longevity or age at sexual maturity, are all important uses of age and growth data (Schramm and Doerzbacher 1982; Hammers and Miranda 1991; Schramm et al. 1992). Slow growth is particularly useful in identifying limiting factors affecting a fish population, such as; limited food resources, over-population, or limiting environmental conditions. Summerfelt (1987) suggested that growth and mortality, along with an estimate of recruitment, provide the three most important rate functions of populations needed to manage fisheries.

Historically, scale measurements have been used for back-calculating fish length at age (Schramm et al. 1992). Recently, as more structures (i.e. otoliths and fin rays) have been used to assess age and growth. Researchers have devoted considerable time toward assessing and improving aging techniques (Hammers and Miranda 1991). Otoliths

(Armstrong 1971; Moring et al. 1981; Lentsch and Griffith 1987; Hubert et al. 1987), scales (Brown and Bailey 1952; Laasko and Cope 1956; Averett and MacPhee 1971; Hubert et al. 1987), and pelvic fin rays (Mills and Beamish 1980; Shirvell 1981) have all been used to age salmonids; however, questions have arisen over the accuracy, precision, and consistency of age determinations among these three structures (Hubert et al. 1987; Campana et al. 1995).

The process of age determination retains an element of subjectivity that contributes to varying degrees of error (Campana et al. 1995). Otoliths, scales, and other calcified structures tend to vary markedly in appearance and size within and among different fish species (Casselman 1987) leading to error in age interpretation (Campana et al. 1995).

Otoliths have been identified to be a better indicators of age than scales in salmonids (Hubert et al. 1987); however, little research substantiates this claim. In high-elevation systems, scales are thought to be inadequate records of age based on the fact that annuli are often not formed the first year due to late spawning times and short growing seasons (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 as annuli are in close proximity to each other and erosion and reabsorption may obliterate annuli in many scales (Alvord

1954).

Description of age and growth of cutthroat trout (*Oncorhynchus clarki*) in the high-elevation tributary streams of the Greybull River and determination of which bony structure is more precise will be valuable information to fishery managers. My objectives were to (1) describe growth of cutthroat trout in the high-elevation streams of the Greybull River drainage, (2) determine if scales or otoliths differed in estimates of age, (3) assess the precision of age estimates of cutthroat trout using scales and otoliths, and (4) validate the use of scales and otoliths to age cutthroat trout.

METHODS

Cutthroat trout were collected from the 17 study streams using Smith-Root Model 12 POW battery back-pack electrofishers. Captured cutthroat trout were weighed (grams) and total length was measured (millimeters). A sample of 1-25 cutthroat trout from each stream was obtained and sagittal otoliths were extracted (Schneidervin and Hubert 1986). Scales were removed from above the lateral line below the insertion of the dorsal fin (Jearld 1983; Knudsen and Davis 1985). A total of 261 fish were collected; otoliths were removed from all fish and scale samples were collected from 100 fish.

In the laboratory, 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 (Hammers and Miranda 1991).

Scales and otoliths from the same 100 cutthroat trout were aged simultaneously by three independent readers, three different times, to determine among and within reader variability (Kimura and Lyons 1991; Campana et al. 1995). Additionally, otoliths from the remaining 161 fish were aged three times by all three readers.

To determine age of individual fish, otoliths and scales were viewed on a video screen using the Wyoming Game and Fish Department's (WGFD) Optical Pattern Recognition System (OPRS; Biosonics 1985). Once the structure had been aged three times by all three readers, the readers agreed upon an age. I then indicated the nucleus, measurement axis, and annuli 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 age. Annuli were identified following criteria set by Jearld (1983) and Mackay et al. (1990).

Several statistical methods have been used to assess accuracy, validity, and precision of age determinations. These include: analysis of variance, paired t-tests, average percent error (Chang 1982), and age difference plots. Campana et al. (1985) suggests that all of these methods have inherent bias and age comparisons are most accurately described with a combination of coefficient of variation and age bias plots.

Coefficient of variation (CV; Chang 1982; Campana et al. 1995) was calculated to determine within and among reader variation in age determination. Coefficients of variation within readers were based on three separate age readings assigned to a single fish by individual readers; while CV's among readers were based on the mean age (from the three readings) assigned each fish by each reader. One-way analysis of variance (ANOVA; Krebs 1989) was used to determine possible differences in mean precision (CV) among readers using the same structure or among readings by individual readers using the same structure. When significant differences were found, Tukey's multiple-comparison test was applied. Two-sample t-tests were used to assess differences in mean precision between structures with a single reader and between structures with all three readers.

Age-bias plots were developed to visually assess differences in aging between readers (Campana et al. 1995). Mean ages determined by one reader were plotted against mean ages determined by a second reader.

Mean total length at age was used to describe absolute growth of fish. The direct proportion method was used to back-calculate total lengths (millimeters) from otolith sections (Lea 1910 *in* Schramm et al. 1992). Back-calculated lengths from scales were calculated using the intercept method (Carlander 1982). An *a* value of 42 mm was used (length at squamation; Brown and Bailey 1952). Two-sample t-tests (Krebs 1989) were used to test for differences in length at age, from otoliths, between hatchery derived and wild populations.

Validation of ages determined from otoliths and scales was based on two year classes of cutthroat trout fry stocked above fish migration barriers in Anderson, Eleanor, and Cow Creeks and the upper Greybull River in 1988 and 1993. In the summer of 1994, these two year classes of cutthroat trout were ages 6 and 1, respectively. Since there is no evidence of natural reproduction in these streams, the stocked year classes provide known-age fish for validation of ageing techniques.

RESULTS

The coefficient of variation (CV) was computed for within and among reader and between structure precision (Table 1). For all three independent readers the CV using otoliths was significantly less than with scales. No differences in CV occurred between readers using scales; however, when using otoliths, reader 2 had a significantly lower CV than readers 1 and 3.

The CV among all three readers after completion of three aging cycles was significantly lower for otoliths (mean CV = 8.4) than scales (CV = 17.3). Differences in CV between two readers were also significantly lower for otoliths than for scales in all comparisons: reader 1 vs. reader 2, reader 1 vs. reader 3, and reader 2 vs. reader 3. No significant differences were found between categories (reader 1 vs. reader 2, etc.) within each structure.

The age bias plots (Figures 1, 2, and 3) were representative of all cases. The plot of reader 1 vs. reader 3 using mean otolith age estimates indicated that there was little bias or difference in ages assigned to otoliths between the two readers. The regression line did not differ significantly at any age from the 1:1 age ratio reference line showing little between reader bias when assigning ages to otoliths. The age-bias plot of reader 1 vs. reader 3 for scales (Figure 2) showed obvious bias between the two readers. Reader 3 consistently aged younger fish to be older and older fish to be younger than reader 1. Again, this plot was representative of the relationships between readers 1 and 2, and 2 and 3. Finally, the bias plot between mean ages assigned to otoliths and scales by reader 1 (Figure 3) indicated that scales consistently were aged younger than the otoliths.

Otolith radius was linearly related to total length of cutthroat trout ($r^2 = 0.837$, $P < 0.001$, Figure 4), with a Y-intercept of -29 mm. This indicated that the use of the direct proportion method of back-calculating lengths at age of cutthroat trout with otolith sections was probably the more valid approach.

Mean back-calculated lengths at age using otoliths for each study stream were computed (Table 2,3). Mean length at age for samples from streams that were stocked were significantly greater than for samples from streams that were not stocked, except for age six.

Back-calculated length at age estimated from scales were larger than those from otoliths (Figure 5). Scale back-calculated lengths were 51 mm greater than otoliths at age 1 and continued to be significantly larger ($P < 0.02$) through age 5; however, the difference declined to only 16 mm at age 6 and was no longer significantly different.

Ages determined by otoliths from the four streams stocked with hatchery fish indicated that two age groups, age 2 cutthroat trout and age 5 and 6 cutthroat trout, dominated the fish stock (Figure 6). However, this age distribution was not apparent using scales (Figure 6) where age 3 and 4 cutthroat trout were most abundant, indicating that the same cutthroat trout aged as 5 or 6 years old with otoliths, were aged younger with scales. Because cutthroat trout fry were stocked into these four streams in 1988 and 1993, the fish were age 1 and 6 at the time of sampling, but neither otoliths nor scales showed this age distribution (Figure 6).

Table 1. Coefficients of variation within and among three independent readers for scales and otoliths.

	Reader precision		
	Scales (n=100)	Otoliths (n=100)	
Reader	Mean CV	Mean CV	P
	Within Reader		
1	13.2[a]	9.55[b]	0.017
2	11.9[a]	5.62[c]	<0.005
3	16.8[a]	8.80[b]	<0.005
	Between Reader		
Among all 3	17.3	8.4	<0.005
1 vs 2	15.1[1,2]	8.52[1]	<0.005
1 vs 3	21.3[2]	8.52[1]	<0.005
2 vs 3	19.3[2]	9.33[1]	<0.005

[a,b,c] Mean coefficients of variation with the same alphabetical superscript were not significantly different (Tukey,s, $P < 0.05$).

[1,2] Mean coefficients of variation with the same numeric superscript were not significantly different (Tukey's, $P < 0.05$).

Figure 1. Age bias plot developed from mean ages assigned to otoliths by readers 1 and 3.

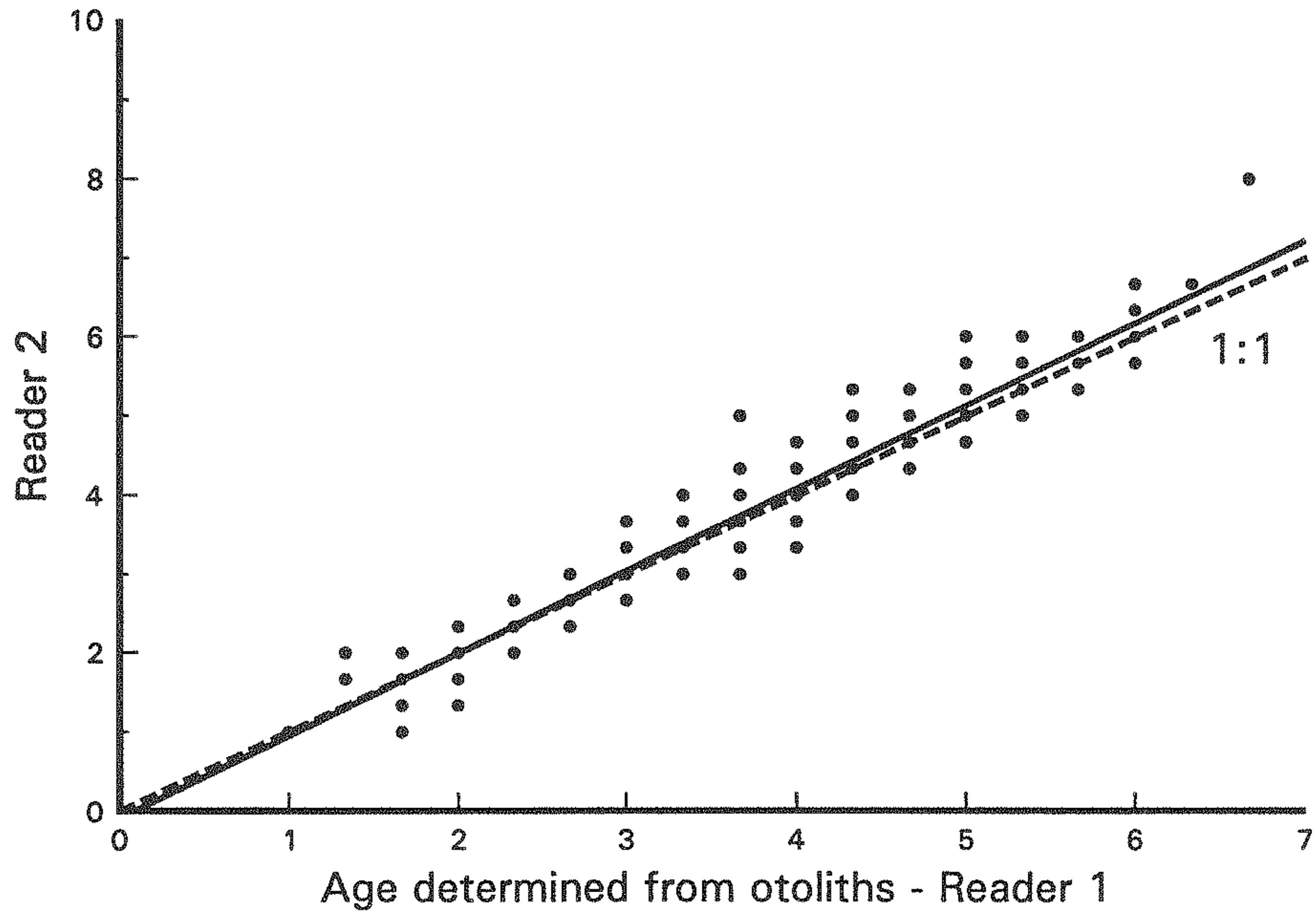

Figure 2. Age bias plot developed from mean ages assigned to scales by readers 1 and 3.

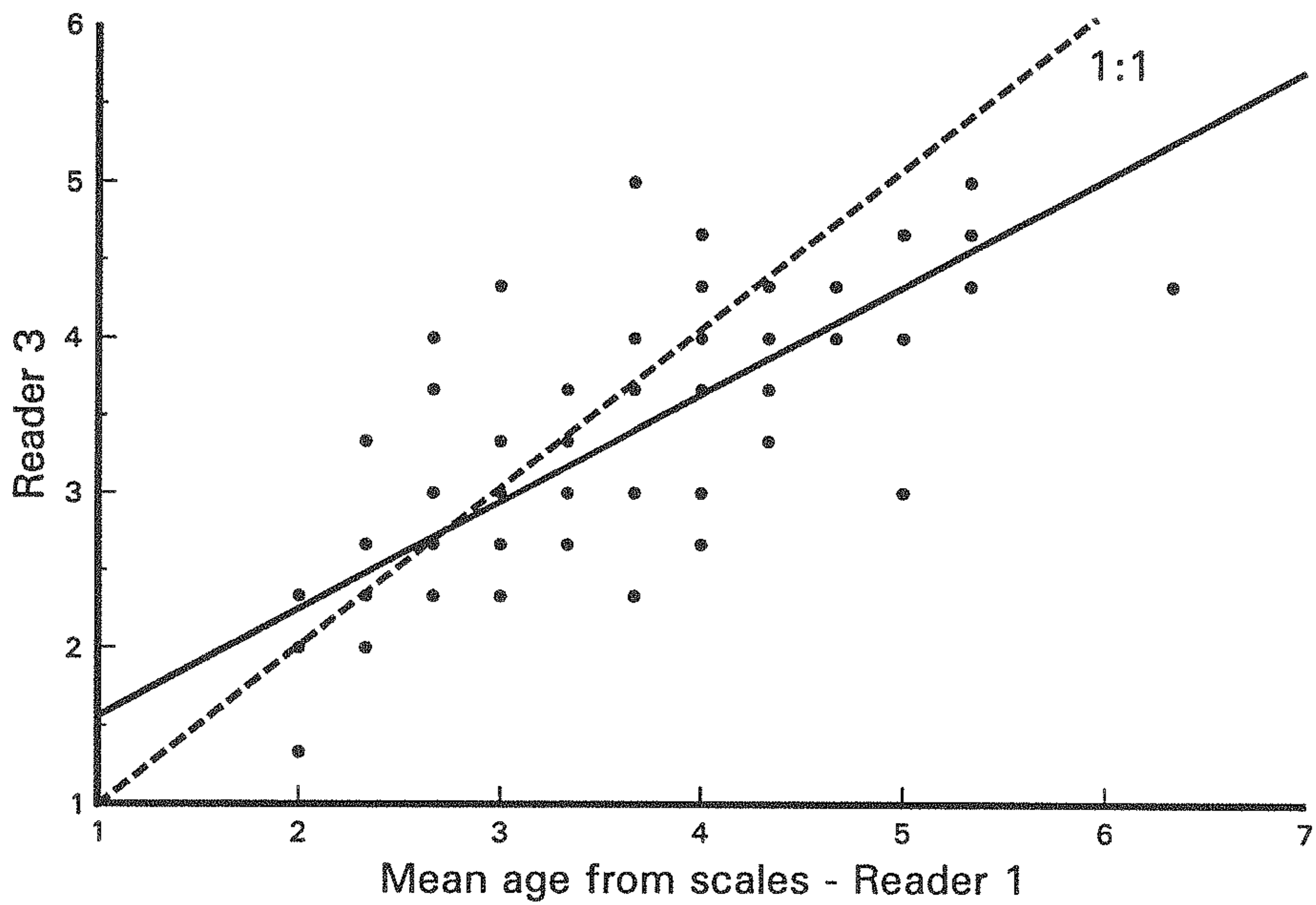

Figure 3. Age bias plot developed from mean ages assigned to scales and otoliths from the same fish by reader 3.

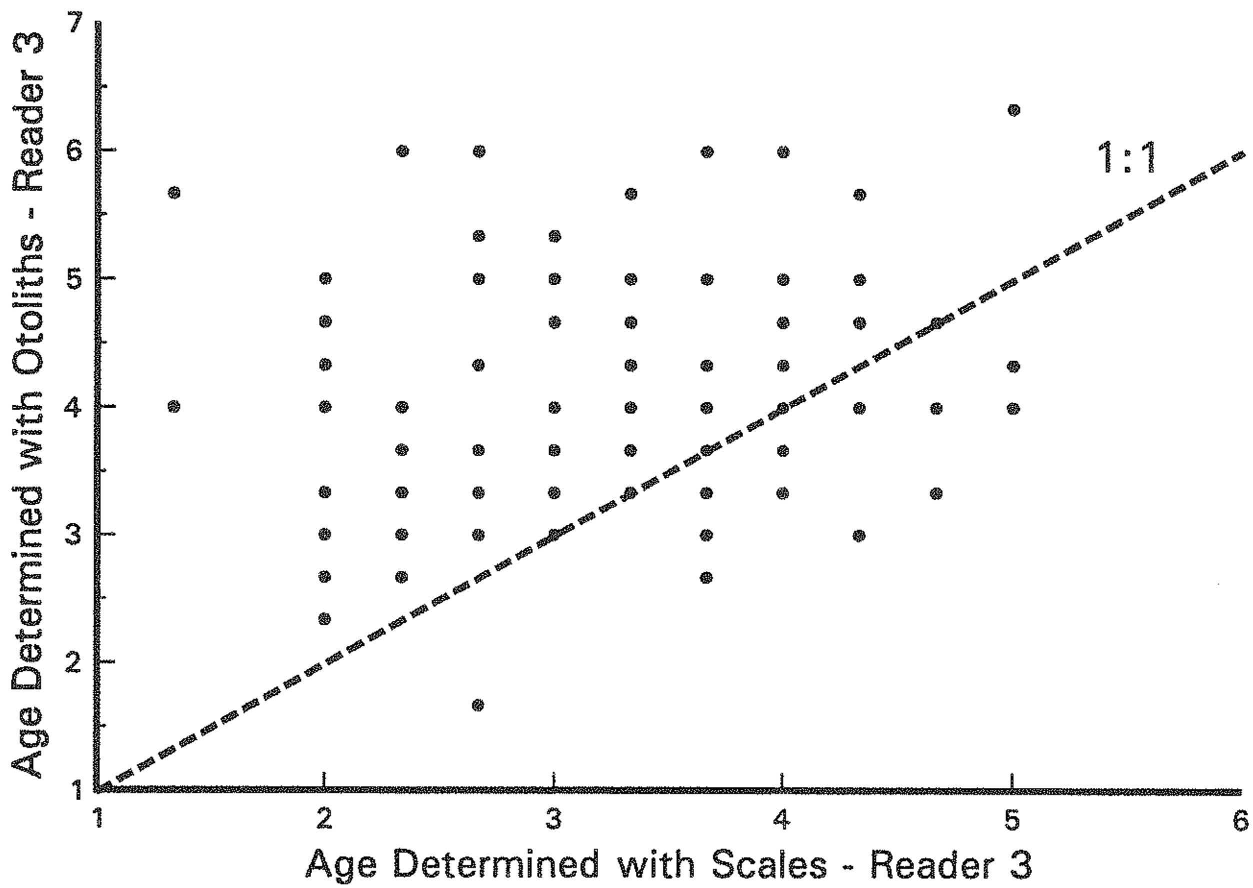

Figure 4. Relationship between mean radius of otolith sections and total length at capture of cutthroat trout from study streams.

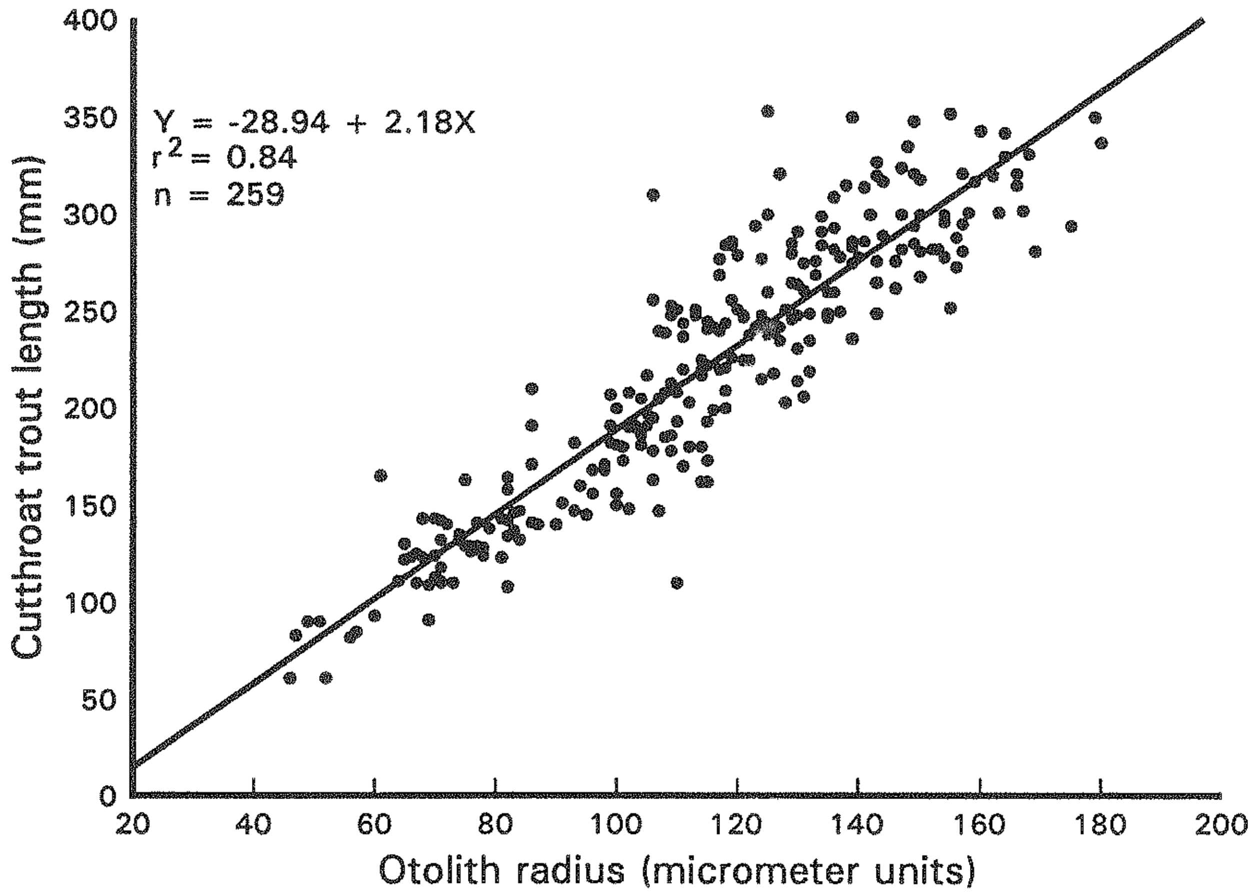

Figure 5. Comparison of back-calculated lengths from scales and otoliths.

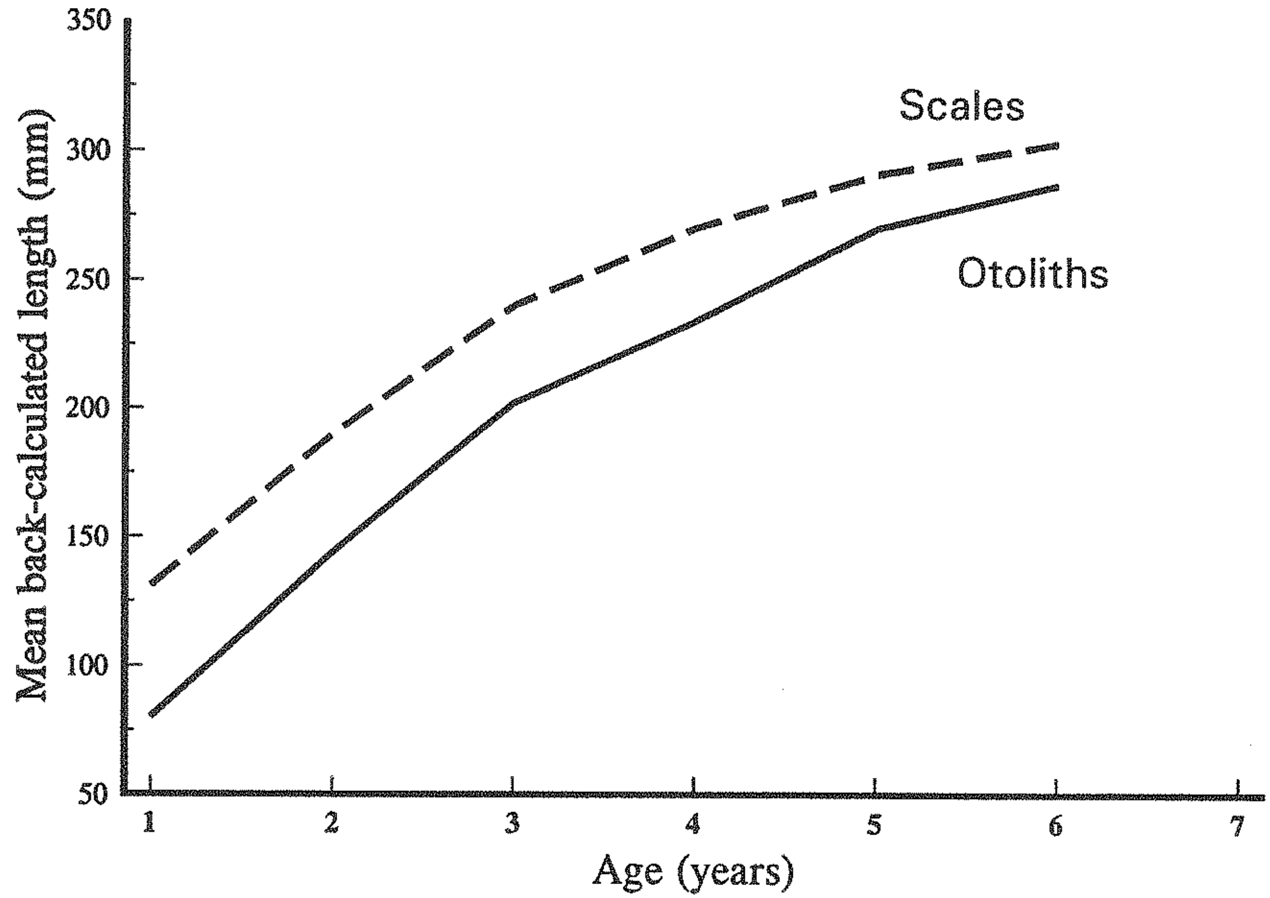

Figure 6. Frequency of cutthroat trout in each age category for scales and otoliths.

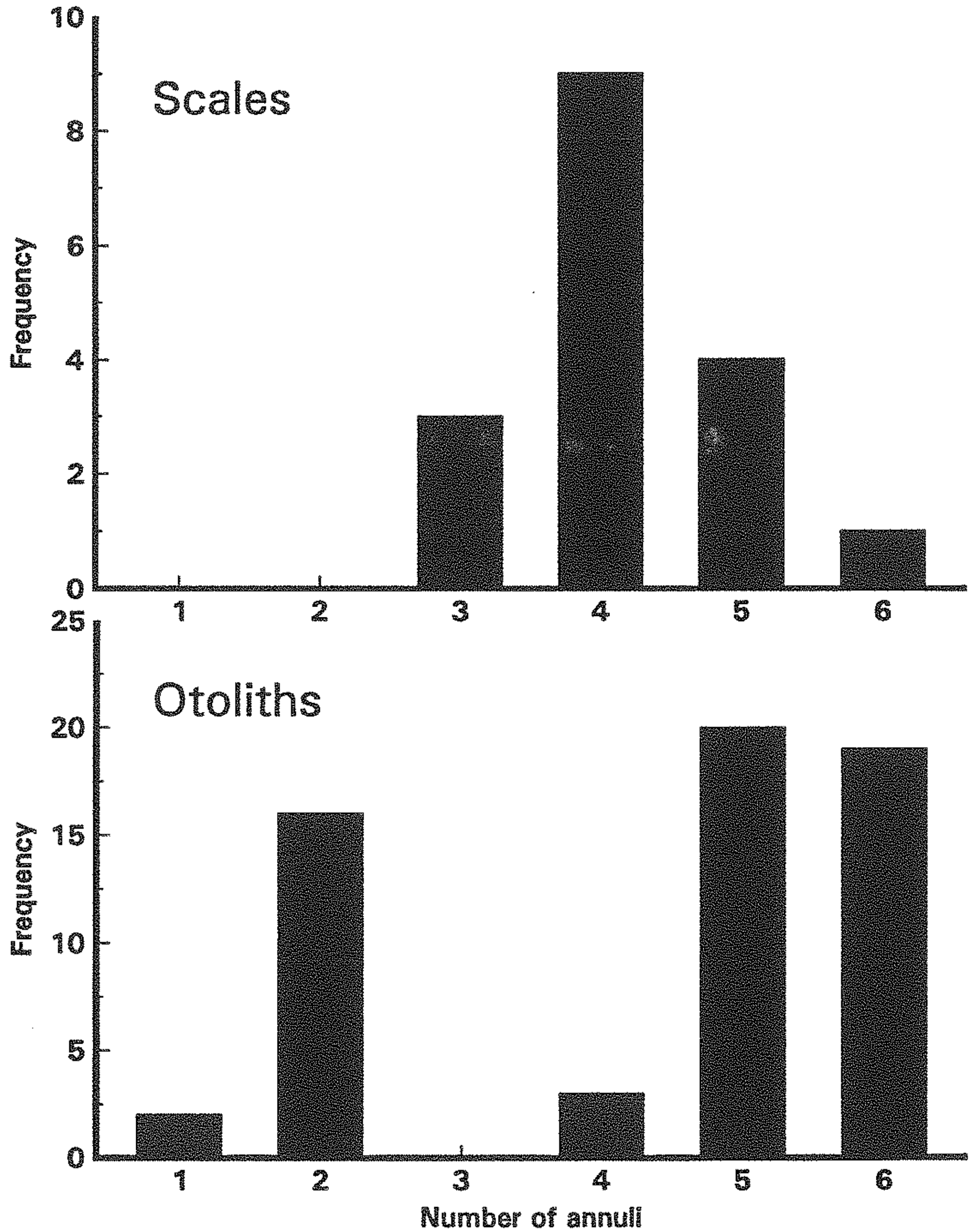

Table 2. Mean back calculated lengths (mm) at age of cutthroat trout from study streams using otoliths.

	Back-calculated length at age (mm)						
Location	**1**	**2**	**3**	**4**	**5**	**6**	**N**
Anderson*	116	160	206	237	259	274	15
Brown	58	119	155	158			17
Chimney	68	142	194	233	255	275	16
Cow*	118	167	251	278	293	304	16
Deer	73	148	205	240	256		14
Dundee	69	139	197				2
Eleanor*	96	136	205	232	256	270	19
U. Greybull*	119	188	252	282	300	322	25
L. Greybull*	68	132	196	226	294	319	10
Jack	56	119	167	201			21
Mabel	71	125	181	228	258		1
Md.F. Wood	64	143	195	234	270		13
Wood	72	149	215	252			21
Pickett	85	157	192	212			17
S.F. Wood	67	136	209	255			18
Venus*a	102	155	222	257	283	268	16
Warhouse	61	128	186	225	244	266	18
Greybull Mean	**80**	**144**	**202**	**234**	**270**	**287**	**259**

* hatchery supported populations
a combination hatchery and wild trout; considered hatchery in means

Table 3. Mean back-calculated lengths at age for hatchery and wild stocks of cutthroat trout in the Greybull River drainage determined from otoliths and scales.

	Back-calculated length at age (mm)						
Method/Group	**1**	**2**	**3**	**4**	**5**	**6**	**N**
Otoliths							
Wild	67	137	190	228	257	276	183
Hatchery	109	158	224	254	274	287	76
All Fish	80	144	202	234	270	287	259
Scales							
Wild	126	184	233	261	291	324	80
Hatchery	149	214	266	284	292	239*	20
All Fish	131	190	240	270	291	303	100

* based on only one cutthroat trout

DISCUSSION

Fisheries scientists utilize measures of growth, mortality, and age structure to compare trout stocks and evaluate management plans. 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 probably more accurate, age estimates than scales for older fish (Hubert et al. 1987; Kozel and Hubert 1987).

I found that otoliths were consistently more precise (lower CV) than scales, suggesting that otoliths from cutthroat trout in high-elevation streams are the better structure for age determination. Age-bias plots (Figures 1, 2, and 3) showed that scales consistently produced lower age determinations than otoliths for individual fish.

Several researchers have found that some stocks of age-0 cutthroat trout, because of late spawning dates and short growing seasons in high-elevation systems, do not grow to an adequate length for scale (Brown and Bailey 1952; Lentsch and Griffith 1987) or annulus (Robertson 1947; Alvord 1954; Laasko and Cope 1956; Bulkley 1961; Averett and MacPhee 1971) formation to occur the first year. Squamation occurs when age-0 cutthroat trout reach 41-44 mm in length (Brown and Bailey 1952); thus, the first annulus in late hatching populations is not laid down on the scale until after the second growing season. Because all three readers consistently aged fish younger using scales, it is probable that cutthroat trout in the Greybull River drainage do not reach squamation length in the first growing season.

Both otoliths and scales were used to back-calculate length at age for cutthroat trout stocks in the Greybull River drainage; however, because the results showed that otoliths were the more precise and less biased of the two structures tested, otoliths were assumed to be the more accurate estimate of age (Hubert et al. 1987). Back-calculated lengths determined from scales were significantly higher than those determined from otoliths at all ages except age 6. At age 1, back-calculated lengths from scales were 51 mm longer than from otoliths. This difference is similar to the squamation length of 41-44 mm suggested for cutthroat trout (Brown and Bailey 1952) and may indicate that the first annulus is absent on scales. Therefore back-calculated length is overestimated the first year of growth using scales and carries over in subsequent years.

Comparisons to other western cutthroat trout stocks (Table 4) were made; however, these age and growth data were obtained using scales. Growth, using either otolith or scale back-calculated lengths at age, of wild cutthroat

Table 4. Length at age (mm) of cutthroat trout from other populations and areas throughout the West using scales and estimates for wild fish using scales and otoliths from the Greybull River (GR) drainage.

	Back-calculated length at age (mm)						
Location	**1**	**2**	**3**	**4**	**5**	**6**	**Citation**
Typical for YSCUT	100	180	240	310	370	410	Varley 1988
Yellowstone Lake, WY	60	140	240	310	350	390	Gresswell 1995
Montana Mean	74	132	198	279	330		Carlander 1969
N.F. Shoshone Streams, WY	67	134	229	304	375	403	Kent 1984
Berry Creek, WY	124	186	256	301			Gulley 1985
Blackfoot, ID	117	213	321	403	442	473	Irving 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
GR scales	**126**	**184**	**233**	**261**	**291**	**324**	**This study**
GR otoliths	**67**	**137**	**190**	**228**	**257**	**276**	**This study**

trout in the Greybull River drainage was slower when compared to other cutthroat trout populations throughout the West (Table 4), especially at older ages. Back-calculated lengths using scales showed similar growth as other high-elevation cutthroat trout stocks in Wyoming (Remmick 1981; Kent 1984; Table 4). However, the slower growth I observed using otoliths in the Greybull River system is more likely to reflect the actual growth in high-elevation mountain systems. The study streams are located in high-elevation, high-gradient systems with harsh environmental conditions the majority of the year. Expected growth would be slower than lower elevation populations were less energy is spent maintaining position and searching for food.

Previous mean lengths from high-elevation systems reported by Remmick (1981) and Kent (1984) probably indicate faster growth than is actually occurring. These data indicate that cutthroat trout in high-elevation systems have slower growth than previous studies suggest.

Hatchery supported stocks (Anderson, Cow, Eleanor, upper Greybull, and Venus) aged with otoliths had significantly higher growth at all ages except age six. Scale age determinations for hatchery stocks indicated similar results (Table 3); however, due to a small sample size, comparisons were not statistically significance. The differences were largest at age one and declined as the cutthroat trout grew older. Hatchery fish would be expected to have significantly faster growth the first year; therefore, it is important to realize the implications of combining growth data. If hatchery stocks are included in age and growth data analysis, length at age of wild cutthroat trout from these high-elevation systems will be over estimated. Finally, with the exception of one 8-year old trout, no cutthroat trout were over 6-years old, indicating that the harsh conditions in the Greybull River drainage limit both growth and survival of older fish.

Age validation is important to test the accuracy of age determination methods (Campana et al. 1995). Cutthroat trout fry were stocked in four study streams, above fish migration barriers, in 1988 and 1993. Because there is no evidence of natural reproduction, the age structure in these streams was 1- (1993 stocking) and 6-year-old fish (1988 stocking). However, otoliths showed a bimodal age distribution dominated by 2-, and 5- or 6-year-old fish (Figure 6). Fish samples aged with scales did not include small fish (< 10 cm total length), but had the largest proportion of fish in the 3- to 4-year-old range. These data indicated that otoliths are probably a more accurate means of ageing cutthroat trout, but they raise further questions about the accuracy of age determination with either otoliths or scales.

Growth data provide valuable information to fishery managers; however, care should be taken to insure that statistics calculated from age data are reliable and accurate. Otoliths appear to provide a more reliable and accurate estimate of cutthroat trout age, but the need to sacrifice the fish is a drawback. Growth in the Greybull River system was slow compared to other wild cutthroat trout stocks in the greater Yellowstone area.

CONCLUSIONS

(1) Otoliths provide more precise estimates of age than do scales for cutthroat trout in the Greybull River system;

(2) Ages determined from scales are consistently lower than from otoliths; therefore, cutthroat trout growth is probably slower than other studies in similar systems have indicated;

(3) Hatchery supported stocks had faster growth at younger ages in the Greybull River drainage; however, growth slowed at older ages; and,

(4) Neither method of age determination provided the expected age distribution in the four streams stocked with hatchery fish, indicating that neither scales nor otoliths may provide accurate age and growth data.

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CHAPTER 6: MANAGEMENT CONSIDERATIONS

Summary

Historically, Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) were the only trout native to the Absaroka Mountain Range of northwestern Wyoming. These high-elevation, volcanically derived mountains are geologically unstable, steep, have large annual flow fluctuations, and are generally lacking fish habitat; thereby producing harsh conditions for trout survival within the Greybull River drainage.

Cutthroat trout currently occupy nearly 45% of the length of perennial streams in the Greybull River drainage. Distributions are influenced by gradient and presence of fish migration barriers. Gradients greater than 9% appear to limit cutthroat trout survival probably due to limited food resources, habitat, and unfavorable energetics. Fish migration barriers block upstream migration of cutthroat trout throughout the drainage; therefore, several areas of apparently suitable habitat located above migration barriers are void of cutthroat trout. Distribution of wild cutthroat trout in the Greybull River drainage is probably similar to historic distributions. Recent Yellowstone cutthroat trout introductions above fish migration barriers may have actually increased cutthroat trout ranges in the Greybull River drainage.

Electrophoretic and meristic evidence indicates that cutthroat trout are not hybridized with rainbow trout in the Greybull River drainage; however, finespotted cutthroat trout genes are present in the fish. The ability of the trout to migrate uninhibited to all parts of the drainage, except above fish migration barriers, suggests that the entire Greybull River drainage should be considered one population composed of finespotted cutthroat trout and Yellowstone cutthroat trout intergrades. Therefore, no genetically pure, wild Yellowstone cutthroat trout can be assumed to persist in the Greybull River drainage.

The overall population structure of cutthroat trout in the Greybull River drainage is best described by a concept of extinction and recolonization of local trout stocks. Environmental oscillation and isolation breaks up the species-population into numerous small, widely separated groups which fluctuate in number and then disappear to be replaced by new individuals from another local stock (Hastings and Harrison 1994). The cutthroat trout in tributary streams in the Greybull River system are similar to these small separated stocks and subject to occasional, localized depletions due to catastrophic events (i.e. debris flows, drought, and flood flows). After depletion or extermination, these localized cutthroat trout stocks in the tributary streams are probably supplemented with fish migrating from the mainstem Greybull or Wood rivers. Thus, cutthroat trout stocks throughout the entire drainage are probably dependent on recolonization from the "source" population in the Greybull or Wood River. Cutthroat trout stocked above fish migration barriers, although subject to periodic depletions or extinction, can not be recolonized by cutthroat trout from other areas. Therefore, they are effectively isolated from other cutthroat trout stocks, but probably need to receive supplemental stockings to maintain cutthroat trout in the stream.

Standing stocks of cutthroat trout in these high-elevation streams were lower than in most streams throughout Wyoming and the West, but similar to streams in Yellowstone Park and adjacent areas (Varley and Gresswell 1988). Cover was found to be the most critical habitat variable influencing standing stocks of cutthroat trout. Boulder pools were the predominate cover type in the study streams. Cover is limited throughout the Greybull River drainage.

Cutthroat trout growth was slow in the Greybull River drainage. Hatchery stocks, although larger at younger ages, appeared to grow slower then naturally recruited cutthroat trout, indicating that hatchery fish are less adapted to the streams in the Greybull River drainage. Use of otoliths was found to be a better method of age determination than use of scales. Scales consistently had larger variation among readers and aged fish younger than otoliths.

<u>Management implications</u>

Cutthroat trout throughout the drainage are hybrids of Yellowstone cutthroat trout and finespotted cutthroat trout. Therefore, the Greybull River drainage cannot be considered as current native Yellowstone cutthroat trout range within Wyoming. The fishery can be managed as a "wild" cutthroat trout fishery, but it should not be considered a "native" population.

Distribution of cutthroat trout and recreational fishing opportunity in the Greybull River drainage could be increased by stocking cutthroat trout above fish migration barriers. Several areas appear to have adequate habitat to support cutthroat trout, including: (1) South Fork of the Wood River; (2) East Fork of the Wood River; (3) the upper Wood River; and (4) the upper Greybull River.

Only pure Yellowstone cutthroat trout should be introduced to the drainage. Although cutthroat trout in the Greybull River drainage are hybridized, genetically pure Yellowstone cutthroat trout stocks should be used in an attempt to dilute the proportion of finespotted cutthroat trout genes within the population (The Yellowstone Cutthroat Trout Working Group 1994). Habitat suitability should be considered before making introductions of cutthroat trout above fish migration barriers.

Debris torrents, droughts, and extremely high flows can deplete or eliminate cutthroat trout populations throughout the drainage. Therefore, periodic supplemental stockings will be required to maintain cutthroat trout stocks isolated above fish migration barriers due to the inability of cutthroat trout to repopulate these areas after catastrophic events.

Due to the harsh environment and limited habitat present in these high-elevation streams, it would be unrealistic to expect to increase cutthroat trout distributions above current levels in the Greybull River drainage. Cutthroat trout probably occupy all presently available habitat in the Greybull system, except areas without fish remaining above fish migration barriers where hatchery stocks could be introduced.

Cutthroat trout from the Greybull River drainage should not be considered for introductions into other river drainages considered as native cutthroat trout habitat. The genetic intergression of these fish with finespotted cutthroat trout does not allow their use as stocks for reestablishing genetically pure, native Yellowstone cutthroat trout in Wyoming.

Due to slow growth and low standing stocks, cutthroat trout stocks may be subject to overharvest if fishing pressure increased. Poor access limits fishing pressure at present; however, localized (i.e. around hunting camps) or increased fishing pressure could significantly impact cutthroat trout standing stocks in the drainage.

LITERATURE CITED

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The Yellowstone Cutthroat Trout Working Group. 1994. Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) management guide for the Yellowstone River drainage. Montana Department of Fish, Wildlife, and Parks, Helena, and Wyoming Game and Fish Department, Cheyenne.

Appendix A. Study locations throughout the Greybull River drainage. Sites indicated with superscript [a] are locations where population estimates and genetic samples were taken. Stocked fish are coded as YSCUT=Yellowstone cutthroat trout, FSCUT=finespotted cutthroat trout, BKT=brook trout, RBT=rainbow trout, and CUT=unidentified cutthroat trout.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
Anderson	1	44.04.974n	109.25.453w	072994	None	Y	Wet
Anderson	2	44.04.816n	109.26.248w	073094	YSCUT 88,93	Y	Wet
Anderson	3	44.04.133n	109.27.238w	073094	YSCUT 88,93	Y	Wet
Anderson	4	44.03.683n	109.29.287w	073094	YSCUT 88,93	Y	Wet
Anderson	5	44.03.116n	109.29.376w	073194	YSCUT 88,93	Y	Wet
Anderson	6	44.02.292n	109.30.946w	073194	None	N	Wet
Anderson	7			073194	YSCUT 88,93	N	Wet
Anderson	8[a]	44.03.925n	109.27.805w	080194	YSCUT 88,93	Y	Wet
Anderson	9	44.05.300n	109.24.738w	080194	None	Y	Wet
Avalanche	1	43.57.790n	109.22.506w	071194	None	N	Trickle
Beaver	1	43.55.276n	109.08.821w	062494	None	N	Dry
Betty	1			081794	None	N	Trickle
Blanchette	1			080994	BKT unknown	N	Dry
Bonnie	1	43.58.698n	109.24.312w	071594	None	N	Wet
Bonnie	2	43.58.458n	109.24.767w	071594	None	N	Wet
Brown	1	43.55.777n	109.06.033w	062194	None	Y	Wet
Brown	2	43.55.430n	109.05.960w	062194	None	Y	Wet
Brown	3	43.54.703n	109.05.851w	062194	None	Y	Wet
Brown	4	43.54.560n	109.05.790w	062194	None	N	Wet
Brown	5[a]	43.55.500n	109.06.072w	062294	None	Y	Wet
Brown Basin	1	43.52.613n	109.17.793w	061894	None	N	Wet
Buckle	1	43.49.667n	109.09.685w	081094	None	N	Wet
Bull Elk	1			081694	None	N	Trickle
Calf	1	43.54.871n	109.21.777w	071194	None	N	Wet
Canyon	1	43.53.760n	109.16.586w	061894	None	N	Wet
Cascade	1	43.52.391n	109.18.038w	061894	None	N	Wet

Appendix A. Continued.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
Cascade	2	43.52.327n	109.17.782w	061894	None	N	Wet
Chimney	1			080994	None	Y	Wet
Chimney	2	43.51.860n	109.06.784w	081094	None	Y	Wet
Chimney	3	43.50.940n	109.06.637w	081094	None	Y	Wet
Chimney	4	43.50.526n	109.06.071w	081094	None	N	Wet
Chimney	5[a]	43.52.009n	109.06.857w	081194	None	Y	Wet
Cow	1	43.57.197n	109.22.700w	071694	YSCUT 88,93	Y	Wet
Cow	2	43.57.242n	109.23.566w	071694	YSCUT 88,93	Y	Wet
Cow	3	43.56.670n	109.23.814w	071694	YSCUT 88,93	Y	Wet
Cow	4	43.55.997n	109.24.163w	071894	YSCUT 88,93	Y	Wet
Cow	5[a]	43.57.482n	109.23.261w	071894	YSCUT 88,93	Y	Wet
Deadman	1	43.49.618n	109.09.624w	081094	None	N	Wet
Deer	1	43.56.040N	109.08.512W	062194	None	(BKT) Y	Wet
Deer	2	43.56.611n	109.09.025w	062194	None	(BKT) Y	Wet
Deer	3	43.57.481n	109.09.725w	062294	None	N	Wet
Deer	4	43.57.293n	109.09.408w	062294	None	(BKT) Y	Wet
Deer	5	43.56.428n	109.08.966w	062294	None	(BKT) Y	Wet
Deer	6[a]	43.56.596n	109.09.012w	062394	None	(BKT) Y	Wet
Dundee	1	43.52.522n	109.10.710w	062594	None	Y	Wet
Dundee	2[a]	43.52.525n	109.11.245w	062594	None	Y	Wet
Dundee	3	43.52.427n	109.11.503w	062594	None	N	Wet
Eleanor	1	44.05.480n	109.28.319w	072994	YSCUT 88,93	Y	Wet
Eleanor	2	44.05.135n	109.28.810w	072994	YSCUT 88,93	Y	Wet
Eleanor	3[a]	44.05.333n	109.26.886w	072994	YSCUT 88,93	Y	Wet
Eleanor	4	44.04.140n	109.26.361w	072994	None	Y	Wet
Eleanor	5	44.04.868n	109.30.354w	072994	YSCUT 88,93	N	Wet
E. Timber	1			082994	CUT 59-64	N	Dry
EF Wood	1	43.50.285n	109.10.270w	081094	None	N	Wet
EF Wood	2	43.49.580n	109.09.710w	081094	None	N	Wet

Appendix A. Continued.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
EF Wood	3	43.48.713n	109.09.206w	081094	None	N	Wet
Francs Fork	1	44.06.042n	109.11.491w	082994	BKT 48;CUT 50-67	Y	Wet
Francs Fork	2	44.04.852n	109.13.062w	082994	BKT 48;CUT 50-67	Y	Wet
Francs Fork	3	44.02.950n	109.14.895w	082994	BKT 48;CUT 50-67	Y	Wet
Francs Fork	4	44.01.706n	109.15.451w	082994	BKT 48;CUT 50-67	Y	Wet
Francs Fork	5[a]	44.003.620	109.14.272w	082994	BKT 48;CUT 50-67	Y	Wet
Galena	1			061894	None	N	Trickle
Greybull	1	43.54.128n	109.19.563w	071094	None	N	Wet
Greybull	2	43.55.486n	109.21.170w	071194	YSCUT 88,93	N	Wet
Greybull	3[a]	43.57.724n	109.22.913w	071694	YSCUT 88,93	Y	Wet
Greybull	4	43.57.663n	109.22.808w	071194	YSCUT 88,93	Y	Wet
Greybull	5	44.01.846n	109.25.612w	072094	FSCUT 72-75	Y	Wet
Greybull	6	43.59.984n	109.24.712w	081494	RBT 15;BKT 16	Y	Wet
Greybull	7[a]	44.02.520n	109.25.248w	081494	FSCUT 72-75	Y	Wet
Greybull	8	44.04.002n	109.25.009w	081694	RBT 15;BKT 16	Y	Wet
Haymaker	1	44.02.820n	109.25.294w	071594	None	N	Trickle
Haymaker	2	44.02.798n	109.24.876w	071594	None	N	Trickle
Horse	1	43.51.650n	109.19.450w	061894	None	N	Wet
Horse	2	43.51.680n	109.19.680w	061894	None	N	Wet
Jack	1	44.06.706n	109.21.090w	080394	CUT 57,58,63,72	Y	Wet
Jack	2	44.05.411n	109.20.925w	080394	CUT 57,58,63,72	Y	Wet
Jack	3	44.04.469n	109.20.842w	080394	CUT 57,58,63,72	N	Wet
Jack	4[a]	44.06.406n	109.21.113w	080394	CUT 57,58,63,72	Y	Wet
Jojo	1	43.56.180n	109.12.750w	061994	CUT 54-56;BKT 35	N	Wet
Jojo	2	43.56.528n	109.13.326w	061994	CUT 54-56;BKT 35	N	Wet
Jojo	3	43.56.820n	109.13.410w	061994	CUT 54-56;BKT 35	N	Wet
Kay	1			082994	None	N	Dry
Last	1	43.58.550n	109.23.299w	071594	None	N	Wet
Last	2	43.58.488n	109.23.258w	071594	None	N	Wet

Appendix A. Continued.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
Mabel	1	43.59.873n	109.24.803w	071194	None	Y	Wet
Mabel	2	43.59.692n	109.24.909w	071194	None	Y	Wet
Mabel	3[a]	44.00.077n	109.25.037w	071194	None	Y	Wet
MF Wood	1	43.55.276n	109.08.821w	062494	None	Y	Wet
MF Wood	2	43.54.578n	109.09.715w	062494	None	Y	Wet
MF Wood	3	43.52.647n	109.10.690w	062594	None	Y	Wet
MF Wood	4	43.51.705n	109.11.265w	062694	None	Y	Wet
MF Wood	5	43.50.520n	109.14.792w	062694	None	Y	Wet
MF Wood	6	43.49.846n	109.15.846w	062694	None	Y	Wet
MF Wood	7[a]	43.52.888n	109.10.472w	062894	None	Y	Wet
Meadow	1	43.55.289n	109.14.610w	062394	CUT 59	N	Wet
Meadow	2	43.55.245n	109.15.106w	062394	CUT 59	N	Wet
No Name	2	43.49.811n	109.16.045w	062594	None	N	Wet
NF Anderson	1	44.03.069n	109.29.609w	073194	None	N	Wet
NF Anderson	2	44.03.210n	109.30.732w	073194	None	N	Wet
NF Cow	1	43.57.218n	109.23.769w	071894	None	N	Trickle
NF Pickett	1	44.11.018n	109.18.411w	062994	None	Y	Wet
NF Pickett	2	44.11.978n	109.18.493w	062994	None	Y	Wet
NF Venus	1	44.58.980n	109.26.645w	071394	None	N	Trickle
Pickett	1	44.06.869n	109.13.025w	062794	BKT 37,48,50	Y	Wet
Pickett	2	44.08.577n	109.14.745w	062794	RBT 49	Y	Wet
Pickett	3	44.10.682n	109.20.166w	062794	CUT 57-59	Y	Wet
Pickett	4	44.11.208n	109.20.796w	062794	BKT 37,48,50	Y	Wet
Pickett	5	44.06.336n	109.12.372w	062894	RBT 49	Y	Wet
Pickett	6	44.11.576n	109.21.216w	062994	CUT 57-59	N	Wet
Pickett	7[a]	44.11.635n	109.21.272w	062994		Y	Wet
Pierce	1			071594	None	N	Dry
Piney	1	44.08.195n	109.21.371w	081794	CUT 52	Y	Wet
Piney	2	44.09.034n	109.22.934w	081794	CUT 52	Y	Wet

Appendix A. Continued.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
Piney	3[a]	44.08.570n	109.22.478w	081794	CUT 52	Y	Wet
Pyramid	1			071194	None	N	Dry
Red	1	44.00.097n	109.24.588w	071094	None	Y	Wet
Red	2	44.00.239n	109.24.302w	071294	None	N	Trickle
Red	3[a]			071294	None	Y	Wet
Slaughter	1	43.48.713n	109.09.206w	081094	None	N	Trickle
Smuggler	1	43.50.551n	109.14.768w	062694	None	N	Wet
Spar	1			061894	None	N	Dry
Steer	1	43.55.348n	109.21.311w	071194	None	N	Wet
Steer	2	43.54.731n	109.22.248w	071194	None	N	Wet
Stuart	1			071594	None	N	Dry
SF Anderson	1	44.03.029n	109.29.444w	073194	YSCUT 88,93	Y	Wet
SF Anderson	2	44.02.687n	109.29.680w	073194	YSCUT 88,93	Y	Wet
SF Anderson	3	44.02.130n	109.29.871w	073194	YSCUT 88,93	N	Wet
SF Anderson	4	44.01.728n	109.30.068w	073194	YSCUT 88,93	N	Wet
SF Wood	1	43.51.737n	109.08.630w	080994	None	N	Wet
SF Wood	2	43.49.695n	109.11.922w	081194	None	N	Wet
SF Wood	3	43.50.632n	109.10.090w	081194	None	N	Wet
SF Wood	4	43.52.283n	109.08.262w	081194	None	N	Wet
SF Wood	5	43.54.217n	109.08.329w	081194	None	Y	Wet
SF Wood	6	43.55.400n	109.06.024w	082794	None	Y	Wet
SF Wood	7	43.58.811n	109.07.677w	082794	None	Y	Wet
SF Wood	8[a]	43.54.519n	109.07.301w	082794	None	Y	Wet
Venus	1	44.00.285n	109.27.882w	071294	YSCUT 88,93	Y	Wet
Venus	2	43.59.652n	109.28.825w	071294	YSCUT 88,93	Y	Wet
Venus	3	43.59.078n	109.29.539w	071294	YSCUT 88,93	N	Wet
Venus	4	44.57.948n	109.30.411w	071394	YSCUT 88,93	N	Wet
Venus	5	44.01.533n	109.25.863w	071994	YSCUT 88,93	Y	Wet
Venus	6	44.01.189n	109.26.250w	071994	YSCUT 88,93	Y	Wet

Appendix A. Continued.

Location	Site[a]	Latitude	Longitude	Date sampled	Stocking records	Trout present	Stream status
Venus	7[a]	44.00.219n	109.27.984w	081594	YSCUT 88,93	Y	Wet
Vick	1	44.05.596n	109.25.464w	081994	None	N	Wet
Warhouse	1	44.05.803n	109.23.459w	080394	None	Y	Wet
Warhouse	2	44.06.702n	109.25.365w	081794	None	N	Wet
Warhouse	3[a]	44.06.087n	109.24.004w	081794	None	Y	Wet
W Timber	1	44.04.118n	109.09.488w	082994	BKT 35-49;RBT 35	Y	Wet
W Timber	2	44.03.447n	109.10.387w	082994	CUT 36, 47-54	Y	Wet
W Timber	3	44.02.193n	109.11.123w	082994	BKT 35-49;RBT 35	N	Wet
W Timber	4	44.01.157n	109.11.113w	082994	CUT 36, 47-54	N	Trickle
W Timber	5[a]	44.03.688n	109.10.962w	082994		Y	Wet
Wood	1	43.51.540n	109.19.770w	061894	FSCUT 72-75	N	Wet
Wood	2	43.55.491n	109.07.166w	082694	RBT 15	Y	Wet
Wood	3	43.55.236n	109.08.127w	082694	FSCUT 72-75	Y	Wet
Wood	4	43.56.010n	109.10.467w	082694	RBT 15	Y	Wet
Wood	5	43.56.111n	109.11.786w	082694	FSCUT 72-75	Y	Wet
Wood	6	43.56.131n	109.12.772w	082694	RBT 15	N	Wet
Wood	7	43.55.530n	109.14.372w	082694	FSCUT 72-75	N	Wet
Wood	8[a]	43.55.890n	109.09.128w	082694	RBT 15	Y	Wet
Yellow	1	43.54.153n	109.19.855w	071094	None	N	Wet
Yellow	2	43.53.661n	109.20.779w	071094	None	N	Wet

Appendix B. Genotypes of individuals and allele frequencies at the genetically variable loci in the samples from the Wood River (W), Greybull River (G), Pickett Creek (P), Anderson Creek (A), Jack Creek (J), Middle Fork of the Wood River (MW), and South Fork of the Wood River (SW). All other loci analyzed, but not listed here, were invariant for the allele characteristic of Yellowstone cutthroat trout. With the exception of sAAT-3,4*, a single number indicates the individual was homozygous for that allele at the locus and two numbers indicates the individual was heterozygous for alleles at the locus. sAAT-3,4* represent an isolocus and for scoring purposes they are treated as a single gene with four instead of two copies per individual.

	Locus and Genotypes		
Fish ID	**sAAT-3,4***	**bGLUA***	**6PGDH***
A178/9	100	100	100
A178/3	110	100	100
A178/1	110	100/70	100
A178/5	100	100	100
A178/13	100	100	100
A178/10	100	100	100
A178/14	90	100/70	100
A178/11	100	100/70	100
A178/7	100	100	100/90
A178/12	110	100	100
A178/2	100	100	100
A178/4	100	100/70	100
A178/15	100	100	100/90
A178/8	100	100	100
A178/6	100	100	100
Allele frequencies	100=0.933 110=0.050 90=0.017	100=0.867 70=0.133	100=0.933 90=0.067

Appendix B. Continued.

Fish ID	Locus and Genotypes			
	sAAT-3,4*	CK-C2*	bGLUA*	sMEP-1*
G247/3	100	100	100	90
G247/1	110/90	100	100	90
G247/18	90/90	100	100	90
G247/15	90	100	100	90
G247/8	90	---	100/70	100/90
G247/2	100	100	100/70	90
G247/9	90	100	100	100/90
G247/17	110/90	100	100	90
G247/14	90	100	100	90
G247/19	90	100	100	90
G247/6	90	100	100/70	100/90
G247/13	90	100	100	90
G247/10	100	100	100	90
G247/7	90/90	100/50	70	90
G247/5	110/90	100	100	90
G247/11	100	100	100	90
G247/16	110/90	100	100/70	90
G247/20	90	100	100	90
G247/4	100	100	100	90
G247/12	90/90	100	100	90
Allele frequencies	100=0.725 110=0.050 90=0.0225	100=0.974 50=0.026	100=0.850 70=0.150	90=0.925 100=0.075

Appendix B. Continued.

Fish ID	Locus and Genotypes		
	sAAT-3,4*	AK-1*	bGLUA*
J194/16	100	100	100
J194/B	110	100	100
J194/23	90	100	100/70
J194/13	90	333/100	100
J194/21	90	100	100
J194/24	100	100	100
J194/H	100	100	100
J194/D	90	333/100	100
J194/14	90/90	333/100	100
J194/18	90/90	333/100	100
J194/C	90	333/100	100
J194/F	100	100	100
J194/22	90	100	100/70
J194/15	100	333/100	100
J194/20	90	100	100
J194/17	100	333/100	100
J194/19	100	100	100/70
J194/E	100	100	100/70
J194/A	100	100	100
Allele frequencies	**100=0.842 110=0.013 90=0.145**	**100=0.816 333=0.184**	**100=0.895 70=0.105**

Appendix B. Continued.

Fish ID	Locus and Genotypes		
	sAAT-3,4*	bGLUA*	IDDH*
P45/2	100	100	100
P35/22	90	100	100
P35/23	90/90	100	100
P45/5	90	100	100
P45/K	110	100/70	100
P45/M	100	100	100
P45/L	110/90	100	100
P35/20	100	100	100
P35/24	90/90	100	100/0
P45/J	110	100	100
P35/19	100	100/70	100
P45/H	110	100/70	100
P45/5	90	100	100
P35/21	100	100	100
P45/I	100	100	100
P35/1	90	100	100
P45/F	90/90/90	100	100
P45/C	90	100	100
P45/3	100	100	100
Allele frequencies	**100=0.766 110=0.053 90=0.171**	**100=0.921 70=0.079**	**100=0.974 0=0.026**

Appendix B. Continued.

	Locus and Genotypes		
Fish ID	**sAAT-3,4***	**AK-1***	**sMEP-1***
MF77/21	110	100	90
MF77/19	100	100	90
MF87/2	90	333/100	90
MF77/24	90	100	90
MF77/23	100	100	90
MF77/16	90/90	100	90
MF77/20	90	100	90
MF87/1	100	100	90
MF77/18	100	100	90
MF77/22	90/90	100	90
MF87/4	90	100	100/90
MF77/17	100	100	90
MF87/5	90	100	100/90
MF87/6	90	100	90
MF87/3	90	100	100/90
Allele frequencies	**100=0.800 110=0.017 90=0.183**	**100=0.967 333=0.033**	**90=0.900 100=0.100**

Appendix B. Continued.

	Locus and Genotypes		
Fish ID	**sAAT-3,4***	**AK-1***	**CK-C2***
SF328/7	110	100	100
SF328/2	90	100	100
SF328/1	90/90	100	100
SF328/6	110	100	100/50
SF328/L	90	333/100	100
SF328/9	90/90	333/100	100
SF328/4	90	100	100
SF328/B	90	100	100/50
SF328/3	110/90	333/100	100
SF328/8	90	100	100
SF328/5	90	100	100
SF328/L	110/90	100	100
SF328/F	90	100	100
SF328/D	90	333/100	100
SF328/A	110/110	100	100
Allele frequencies	**100=0.667 110=0.100 90=0.233**	**100=0.867 333=0.133**	**100=0.933 50=0.067**

Appendix B. Continued.

	Locus and Genotypes			
Fish ID	**sAAT-3,4***	**AK-1***	**bGLUA***	**sMEP-1***
W308/4	90	100	100	90
W308/16	90	100	100	90
W308/15	90	100	100	90
W308/5	100	100	100	100/90
W308/19	90	100	100	90
W308/3	90	100	100	90
W308/6	90/90	100	100/70	90
W308/13	90	100	100	90
W308/2	100	100	100	90
W308/22	90	100	100	90
W308/10	100	100	100/70	90
W308/7	110/90	100	100/70	90
W308/11	90	100	100	90
W308/1	110	100	100	90
W308/12	90	333/100	100	90
W308/8	90	100	100	90
W308/14	90	100	100	90
W308/AP	90	100	100	90
W308/9	100	333/100	100	90
W308/17	100	100	100	90
Allele frequencies	**100=0.788 110=0.025 90=0.188**	**100=0.950 333=0.050**	**100=0.925 70=0.075**	**90=0.975 100=0.025**