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BIG HOLE RIVER ARCTIC GRAYLING RECOVERY PROJECT:

ANNUAL MONITORING REPORT 1993

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Fluvial Arctic Grayling Workgroup

and

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ABSTRACT

This annual monitoring report of the Big Hole River Arctic grayling recovery project contains the results of discharge and water temperature, population, and Axolotl brood reserve monitoring. Spring and summer rains provided ample flow through the summer and maintained moderate water temperatures. The spawning population was composed of over 80% age 3+ spawners. Although the spawning population was substantial, recruitment was poor in 1993. An increase in discharge as larvae hatched and emerged probably limited survival of young. The population remained stable in the McDowell-Wisdom section, which was estimated to be 32 ± 22 (95% confidence interval) age 1+ grayling per mile. The entire grayling population in the 66 miles of river above Divide was estimated to be 2,549 ± 2,078 age 1+ grayling. The 1992 year class was well represented in the population and will provide the majority of spawners in the next 3 years. Gametes were collected from Axolotl Lakes for addition to the brood stock and to provide fish for introductions. Eggs were stripped from 40 females and fertilized by 2 to 4 males each. Approximately 3,000 yearling grayling were planted in the brood lake to supplement the aging brood reserve at Axolotl Lakes.

ACKNOWLEDGEMENTS

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INTRODUCTION

The Arctic Grayling Recovery Program was initiated in 1991 to coordinate efforts to restore and protect the only remaining native fluvial Arctic grayling (<u>Thymallus arcticus</u>) population in the contiguous United States (Byorth 1991). After declining in abundance between 1984 and 1989, the population in the Big Hole River stabilized at approximately 30 age I+ grayling per mile (Byorth 1993). Reasons for the decline in abundance are unclear. Research to elucidate ecology and life history of grayling in the Big Hole Basin began as early as 1979 (Byorth 1991). Primary objectives of current investigations, under direction of the Fluvial Arctic Grayling Workgroup, are:

I. Define ecological factors limiting the abundance of Arctic grayling in the Big Hole River drainage,

II. Monitor population trends, enhance abundance, and expand the range of fluvial Arctic grayling, and

III. Educate the public to increase awareness and support for grayling restoration.

Specific objectives of 1993 research and monitoring included:

A. Monitor population abundance and distribution in the Big Hole basin,

B. Monitor water temperature and discharge in the Big Hole River,

C. Monitor the reserve stock and collect gametes from grayling at Axolotl Lakes,

D. Test thermal tolerances of grayling and compare with thermal regimes recorded in the Big Hole River,

E. Analyze the interspecific interactions between grayling and non-native species,

F. Conclude analysis of the effects of angling on the grayling population,

G. Characterize and quantify grayling habitat in the Big Hole River, and

H. Analyze seasonal movements and habitat usage of grayling in the Big Hole Basin.

Progress on objectives A, B, and C, for data collected between March and November, 1993 are reported herein. Results of objective D (Lohr et al., In Press) are included as Appendix A. Results of objectives E, F, G, and H will be reported separately.

METHODS

Discharge and Water Temperatures

Discharge and water temperature were monitored as described in Byorth (1993). The U.S. Geological Survey (USGS) gage located at Wisdom recorded temperature and discharge. Data provided by USGS and used in analyses were preliminary. Omnidata DP212 digital thermographs recorded water temperatures at 120 min intervals at 4 locations along the Big Hole River (Figure 1). Water temperature was recorded on memory chips which were replaced approximately every 85 days. Data were downloaded into DBase III+ files (Ashton-Tate, Scotts Valley, CA) and analyzed using DBase programs.

Population Surveys

We used electrofishing to sample grayling and trout in the Big Hole River and its tributaries. A drift boat or Coleman Crawdad mounted mobile anode system was used, consisting of a 4,000 watt AC generator with a Leech or Coffelt Mark XXII rectifying unit to convert output current to DC. The system was fished downstream as trout and grayling were netted and retained in a live well for processing. After anesthetizing fish in an ethyl 4-aminobenzoate or MS-222 bath, we measured them to the nearest 0.1 inches in total length and weighed them to the nearest 0.01 lb. We collected scale samples for age determination, clipped a fin, noted presence of hooking wounds, and tagged each grayling with an individually numbered and color-coded VI tag (visible implant, Northwest Marine Technology, Inc.). We released fish after they recovered from anesthesia. We calculated population estimates with mark-recapture ratios using the Chapman Modification of the Peterson mark/recapture model (Chapman 1951, Vincent 1971). Confidence intervals are reported at $\alpha=0.05$.

We conducted spawning surveys in four sections of the Big Hole River: McDowell, Wisdom, North Fork, and Pintlar Creek to Squaw Creek. Additionally, sections of Deep Creek, Fishtrap Creek, La Marche Creek, Rock Creek, and Swamp Creek were electrofished. An electrofishing crew made only one pass in each section and tributary between April 7 and 30, 1993. Sex and spawning condition of each grayling were determined: as gravid female grayling were captured, surveys were discontinued to minimize affects of electrofishing on spawners and larvae.

To refine our estimate of the abundance of Arctic grayling population in the Big Hole River, we electrofished from the head of the McDowell section downstream to the bridge above the Butte Water Intake, approximately 66 miles. We marked and recaptured grayling and brook (Salvelinus fontinalis), rainbow (Oncorhynchus mykiss), and brown trout (Salmo trutta) from September 1 to October 7, 1993. We calculated population estimates for the entire 66 mile reach as well as for the McDowell and Wisdom sections. We also surveyed Deep Creek, La Marche Creek, Swamp Creek, and Fishtrap Creek for young-of-the-year (YOY) grayling.

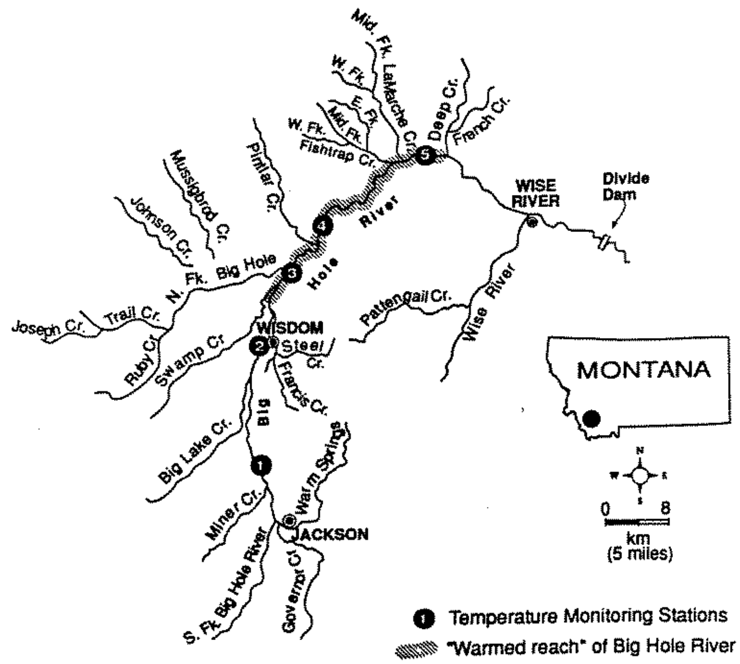

Figure 1. Map of Big Hole River showing thermograph stations and USGS gaging station. Stations are numbered: 1, Peterson Bridge; 2, Wisdom (USGS gage); 3, Buffalo Ranch; 4, Christianson's; and 5, Sportman's Park.

Axolotl Lake Brood

The Axolotl Lake brood was established in 1988 to provide a reserve population of Big Hole River grayling separate from that held at the Bozeman Fish Technology Center (FTC). To maintain a representation of the 1988 year class in the brood reserve, we collected gametes in May 1993. We captured grayling using 2 fyke nets set at different locations in the lake. Net leads were run from shallows and fykes were set at approximately 12 ft deep. We operated the nets overnight. Grayling were removed from the fykes and sorted by sex. Each sex was retained in a 48"x48"x60" live car placed in the lake. A sample from each catch was measured, weighed, and spawning condition was noted. Personnel from USFWS Ennis National Fish Hatchery (ENFH) spawned grayling. Eggs were stripped into a vial and fertilized with milt collected from at least 2 males using an aspirator. After several minutes we rinsed the eggs with water to remove excess milt. The grayling were allowed to recover and were released. Fertilized eggs were transported to the FTC for rearing. A sample of spawned grayling was taken for disease analysis. Water temperatures were monitored, at approximately 8 ft deep, with an Omnidata DP212 thermograph.

RESULTS

Discharge and Water Temperature

The Big Hole River hydrograph in 1993 reflected near normal snowpack and consistent precipitation between April and September (Figure 2). Flows fluctuated between moderate extremes: instantaneous peak flow was 1,830 cfs on June 17 and minimum flow was recorded on September 30 at 64 cfs. In contrast to the extreme drought conditions of 1992, flows peaked at 610 cfs on June 17, 1992 and reached 3.3 and 3.9 cfs on May 26 and September 4, 1992, respectively. While discharge was stable during spawning, a pulse in flow occurred just after the approximate period in which grayling fry should have emerged from spawning gravels. Discharge during the predicted emergence period of May 11 - 15 averaged 388 cfs (range: 264 - 537). Flows increased to a mean of 682 cfs (range: 648 - 746) between May 16 and 23. This increase in flow may have reduced survival of the vulnerable larvae.

Consistent flows and precipitation moderated water temperatures in 1993. Water temperatures did not approach the extremes recorded in 1992. The maximum temperature of 71°F was recorded at the Wisdom Bridge (Station 2, Figure 1) on June 20, 1993. However, maximum mean daily temperatures were higher at downstream stations than at Wisdom (Table 1). Temperatures in the study reach did not approach lethal limits as they did in 1992 (Lohr et al. Appendix A).

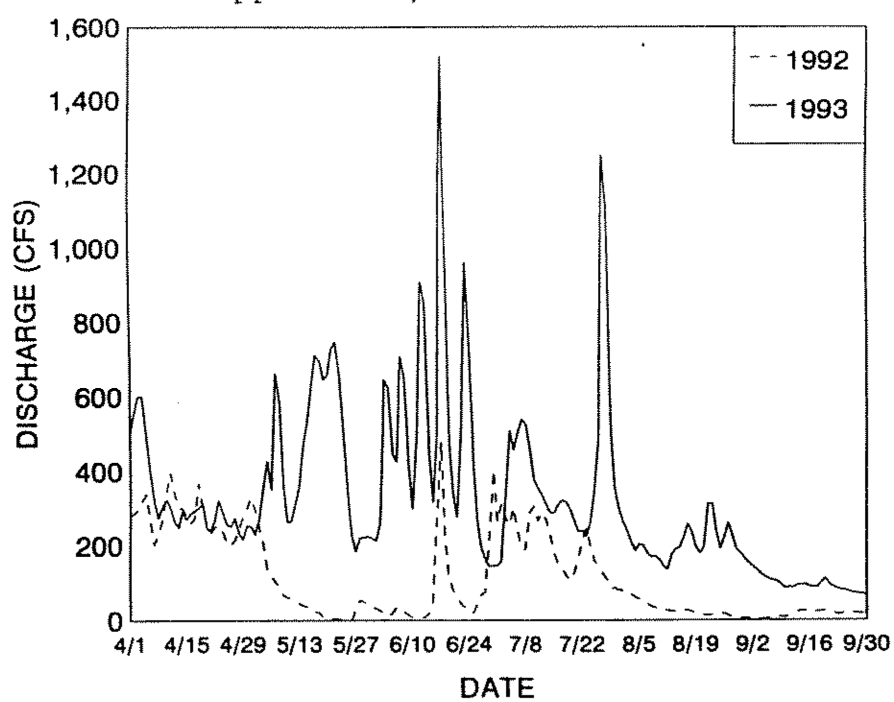

Figure 2. Mean daily discharge of the Big Hole River at the Wisdom gage station April through October, 1992 and 1993.

Table 1. Maximum daily (T_{max}) and maximum mean daily water temperature (°F) at thermograph stations in the Big Hole River 1992 and 1993.

Thermograph Station	T_{max} (°F)		Mean T_{max} (°F)	
	1992	1993	1992	1993
Peterson Bridge	71.6	68.9	64.0	62.1
Wisdom Bridge	75.9	71.1	66.4	64.6
Buffalo Ranch	77.0	70.7	69.1	65.6
Christianson	81.5	69.8	68.5	65.0
Sportman's Park	79.7	68.0	70.6	63.9

Population Monitoring

Spawning and Recruitment

We surveyed the spawning run to characterize the mature grayling population. To minimize any potential effects of electrofishing on spawning, surveys were discontinued prior to the onset of spawning. Based on sex ratios and observations of ripe females, the peak of spawning probably occurred between April 24 and 28. During this period, discharge averaged 260 cfs (range: 213 - 310) at the Wisdom gage and mean daily water temperatures ranged from 43.1 to 45.7°F.

We captured 134 age 2 and older grayling: 105 in the Big Hole River and 29 in tributaries. The sex ratio was 1.17 males per female. The spawning population was composed of 11.9% age 2, 38.9% age 3, 41.9% age 4, and 6.2% age 5 grayling. Age 3 and older grayling comprised 87% of the spawners, which is a slight increase over the previous high observed in 1992 (Figure 3). The high proportion of older spawners, as in 1992, would be expected to provide good recruitment.

Tributaries of the Big Hole River appear to substantially contribute to recruitment. Evidence of spawning was gathered in Deep, La Marche, Fishtrap, Swamp, and Rock creeks (Table 2). Yearling grayling (1992 cohort) were captured in all the above streams. Evidently, juvenile grayling remained in their natal stream through their first winter. Adult grayling in spawning condition were captured in Swamp and Rock creeks. However, we sampled La Marche, Deep, and Fishtrap creeks almost two weeks prior to the estimated peak of spawning; consequently, sampling may have preceded spawning migrations. No adult or yearling grayling were captured in Governor Creek.

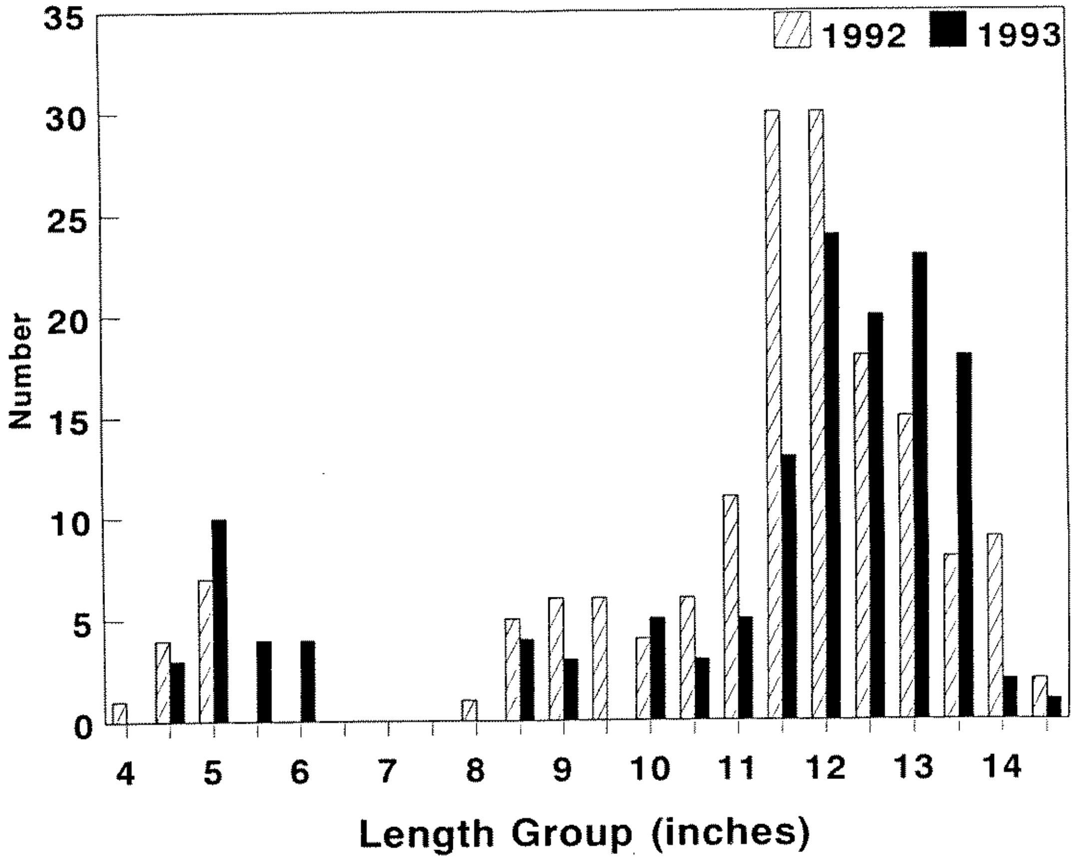

Figure 3. Length frequency distribution of Arctic grayling captured in Big Hole River spawning surveys 1992 and 1993.

Table 2. Tributaries and study sections of the Big Hole River sampled during Spring 1993 and number of yearling and age 2 and older Arctic grayling captured.

Stream/Section	Date	# Age 1	# Age 2+
Deep Creek	4/12/93	17	3
La Marche Creek	4/13/93	63	0
Fishtrap Creek	4/13/93	2	1
Swamp Creek	4/28/93	6	16
Governor Creek	4/29/93	0	0
Rock Creek	4/30/93	1	4
Wisdom Section	4/20-21/93	4	56
McDowell Section	4/19/93	3	4

Recruitment to the 1993 year class appears to have been poor, in spite of an ample spawning population. The relative abundance of YOY grayling, as indicated by catch per unit effort in Fall surveys of the McDowell and Wisdom Sections, was the lowest since 1987 (Table 3). Discharge patterns may have limited spawning success. By calculating degree days to hatching as outlined in Kratt and Smith (1977) and Wojcik (1955), we estimate that hatching occurred between May 10 and 12 and larvae emerged between May 13 and 15. Immediately after emerging, young grayling were exposed to an 87% increase in mean daily discharge.

Fewer YOY grayling were captured in fall surveys and were more evenly distributed than in 1992 (Figure 4). Highest numbers of YOY grayling were captured in the Wisdom East (Steel Creek) channel. Higher flows may have forced emergent larvae from traditional rearing areas and resulted in the dispersed distribution. However, higher flows throughout the fall period may have simply decreased the efficiency of sampling gear.

Table 3. Catch rates (catch-per-effort CPE) of Young-of-the-Year (YOY) grayling captured in the McDowell and Wisdom sections of the Big Hole River, 1983 - 1992.

Year	McDowell Section			Wisdom Section		
	# YOY	# Runs	CPE	#YOY	# Runs	CPE
1983	---	---	---	2	6	0.33
1984	---	---	---	5	7	0.71
1985	0	3	0	0	3	0
1986	145	4	38.2	---	---	---
1987	3	1	3.0	0	1	0
1988	---	---	---	---	---	---
1989	178	2	89.0	90	2	45.0
1990	58	2	29.0	98	4	24.5
1991	10	2	5.0	41	2	20.5
1992	42	2	21.0	83	4	20.75
1993	2	2	1.0	31	4	7.75

Population Estimates

The grayling population of the Big Hole River remains stable at levels similar to those estimated from 1989 to 1992. An estimated 32 ± 22 age 1+ grayling per mile resided in the combined McDowell and Wisdom Sections (M=48, C=38, R=5). Yearling grayling (7.0-10.9 inches) were estimated at 27 ± 22 per mile and age 2+ (≥ 11.0 inches) at 5 ± 4 per mile. The length frequency distribution indicates the strength of the 1992 year class and poor recruitment in 1991 (Figure 5).

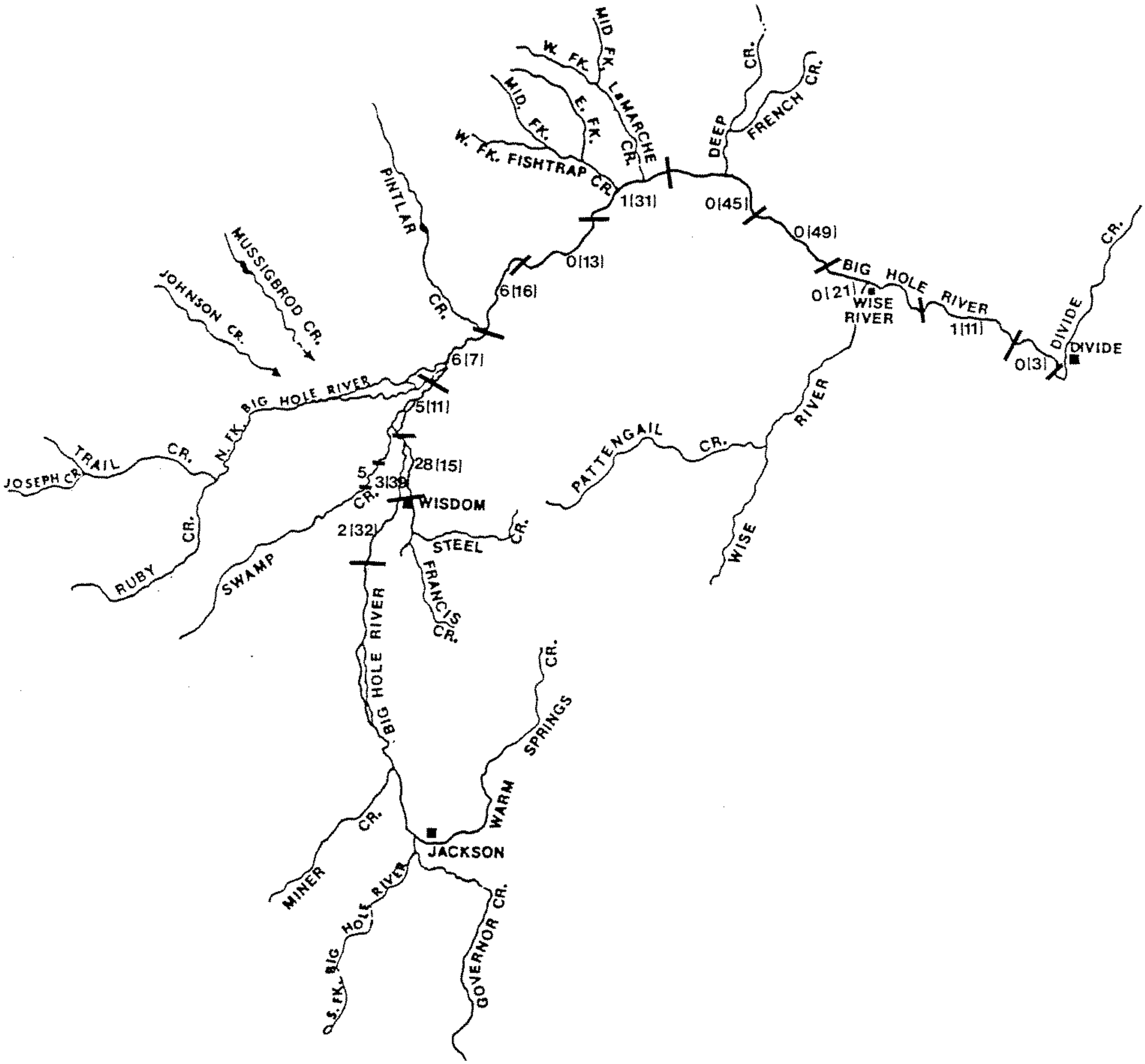

Figure 4. Map of number of young-of-the-year and age 1+ grayling (in parentheses) captured per reach in the Big Hole River, Fall, 1993.

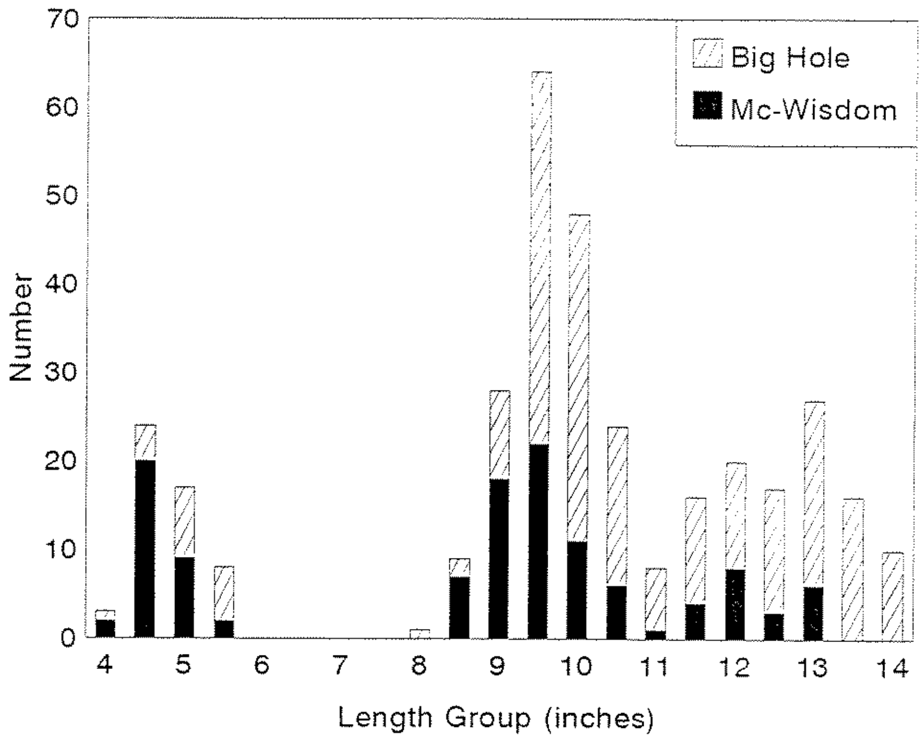

Figure 5. Length frequency distribution of Arctic grayling captured in Fall electrofishing surveys of the Big Hole River, 1993.

While the Wisdom and McDowell sections provide an index of grayling abundance, it was necessary to attempt to quantify the entire population of grayling in the Big Hole River between the McDowell Section and Divide. We captured a total of 283 age 1+ grayling in two electrofishing passes through the 66 mile reach. Based on mark/recapture ratios we estimate the population comprises 2,549 ± 2,078 yearling and older grayling. If we assume that the grayling are uniformly distributed, then the "standing crop" of grayling is approximately 39 ± 31 per mile. Parameters of the estimate are provided in Table 4.

Table 4. Parameters used to estimate grayling abundance in a 66 mile reach of the Big Hole River, Fall 1992. Parameters are M = number marked, C = number captured in second pass, R = number marked fish recaptured, N = estimated number, and 95% CI = confidence interval.

Length Group (inches)	M	C	R	N ± 95% CI	N/mile ± 95% CI
8.0 - 10.9	90	85	3	1,956 ± 1,674	30 ± 25
≥ 11.0	52	64	5	573 ± 404	9 ± 6
Total	142	149	8	2,529 ± 2,078	39 ± 31

The distribution of grayling captured during the surveys indicated that the McDowell and Wisdom Sections still provide key habitats which support relatively high densities of grayling (Figure 4). However, nearly equal numbers of grayling were captured in the reach between Sawlog Creek and Eastbank. Estimated abundance in this reach was 31 ± 26 grayling per mile. Stable mid-summer flows may have allowed grayling to distribute themselves into habitats less suitable during recent drought years.

Yearling grayling composed approximately 61% of grayling handled during fall surveys. Again, the 1991 year class is predominant in the length frequency distribution (Figure 5).

We documented a fall run of grayling into Deep Creek. On October 7, we captured 96 grayling in a 1.5 mile reach. When we returned on October 17, we captured only 9: 2 of which were previously marked.

Axolotl Brood Reserve

The brood reserve at Axolotl Lakes provides juvenile grayling for FTC brood stock, introductions, and experiments. In May 1993, we collected gametes for these purposes. We captured 395 grayling: 91 females and 314 males to provide gametes. The disparity in sex ratio is due to the trapping before the majority of females entered spawning condition. The Axolotl brood reserve is 6 years old and average length was 13.5 inches (range: 9.4 to 14.6 inches). Females began to enter spawning condition by May 14, but the majority were not ripe until May 24. Females were ripe approximately 1 week later than in 1992, when water temperatures averaged between 50.2 and 51.4°F. We spawned 2 to 5 males on each of 40 females and transported the fertilized eggs to FTC in Bozeman.

Axolotl reserve grayling are nearing the end of their life span. To maintain a reserve population, approximately 3,000 yearling grayling were planted into the brood lake on July 19. Half of the plant was an F_1 generation of wild Big Hole grayling spawned in 1992, which were marked with an adipose clip. The remainder were progeny of the Axolotl brood spawned in 1992 and marked with a permanent left pelvic fin clip.

DISCUSSION AND MANAGEMENT IMPLICATIONS

The Arctic grayling population of the Big Hole River remains stable at low numbers. The population has been stable since 1989 at approximately 30 per mile. The decline between 1984 and 1987 was coincidental with record floods followed by severe drought (Byorth 1993). Water supplies from Spring through Fall 1993 provided ample flow due to near average snowpack and above average spring and summer precipitation. Minimum flow in 1993 was approximately 20 times greater than minimums in 1988 through 1992. Expected benefits of improved flows include better survival of adult grayling, increased availability of habitat, improvement in channel morphology during prolonged flushing flows, and moderate mid-summer water temperatures. Mid-summer water temperatures did not reach or exceed lethal limits as defined by Lohr et al. (Appendix A) as they had in 1992.

One potential negative impact of higher flows was limiting recruitment. Because Arctic grayling larvae have poorly formed fins and are weak swimmers when they emerge, they are susceptible to high flows (Nelson 1954, Lee 1985). Clark (1992) documented a significant negative correlation between discharge and grayling recruitment in the Chena River in Alaska. Although the direct impacts are not well understood, Lee (1985) found that grayling larvae may be displaced by high velocity flows or can be stranded in high water habitats as water levels recede. Shepard and Oswald (1989) theorized that well-above average spring flows result in weak year classes in the Big Hole River. During the period that grayling hatched and emerged in 1993, mean daily discharge nearly doubled. Poor catch-per-effort of YOY in the McDowell-Wisdom sections reflects poor recruitment in spite of an ample spawning run. Similar numbers and age distribution of spawners were observed in 1992, but flows were extremely low during hatching and emergence and excellent recruitment was observed (Byorth 1993). Research into the relationship between discharge and recruitment should be pursued.

The apparent relationship of discharge patterns and recruitment has important implications. Erratic recruitment has been observed throughout the last decade, especially as the population declined. The Big Hole grayling population has stabilized at low levels because of alternating good and poor recruitment years. However, the potential for several consecutive poor recruitment years is not unrealistic and would have drastic consequences for the population. Conversely, several consecutive years of good recruitment would probably result in an increase in abundance of grayling. Because this population is at risk, supplementation of YOY or yearling grayling should be pursued especially during poor recruitment years. Benefits of supplementation could be threefold: assessment of recruitment from YOY to yearling, supplementation would increase the population, and decrease the probability of extinction. Progeny of the Big Hole grayling brood stock will be genetically suitable for planting into the Big Hole River by

1995. Supplementation of wild grayling with brood grayling should begin as soon as possible thereafter.

Grayling spawning in tributaries plays an important role in recruitment to the population. We documented indirect evidence of spawning in Rock, Swamp, La Marche, Fishtrap, and Deep creeks. The contribution to overall recruitment may be substantial. Liknes and Gould (1987) found no evidence of spawning in Deep or Fishtrap creeks, but found age 1+ grayling in La Marche Creek. Shepard and Oswald (1989) documented spawning in Steel (Wisdom East Section and above Highway 43 bridge), Big Lake (McDowell Section), Swamp, Rock, and Sand Hollow creeks. Although the McDowell and Wisdom areas still support the majority of spawning, tributaries below Squaw Creek (Fishtrap, La Marche, and Deep Creeks) appear to contribute to recruitment. Whether grayling are pioneering new areas or are reentering traditional spawning areas is unclear and should be investigated.

The erratic nature of recruitment is evident in the last several years (Table 3). Strong year classes were observed in 1990 and 1992. The 1990 cohort carried through well and should contribute to spawning in 1994. The abundant 1992 year class will begin to spawn in 1994 but will not contribute greatly until 1995 and 1996 when they are fully mature. Because the 1993 cohort is weak, it may offset any major increases in population abundance.

Monitoring the grayling population will continue to be important to the restoration program. Until 1993, population monitoring was conducted in the McDowell and Wisdom sections in fall. Results of the large-scale population survey indicate that the McDowell and Wisdom sections provide a good index of the population as a whole. Although grayling are distributed patchily throughout the basin, a per mile estimate for the 66 mile reach is similar to that of the McDowell-Wisdom sections. Monitoring of the McDowell-Wisdom section also provides an index of recruitment and should be continued.

The fall migration of grayling into Deep Creek is a curious phenomenon. We documented substantial numbers of grayling entering and leaving Deep Creek during a short period. They may be following spawning brook or brown trout, or mountain whitefish (<u>Prosopium williamsoni</u>). Byorth (1991) found that radio-telemetered fish moved extensively during mid-October, the same period in which grayling moved into Deep Creek. A local landowner has observed this migration in the past (P. Ralston pers. comm. to B. Shepard). Further investigations of fall migrations will be conducted.

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APPENDIX A

Lohr, S. C., P. A. Byorth, C. M. Kaya, and W. P. Dwyer. In Review. High temperature tolerance of fluvial Arctic grayling and comparisons with summer water temperatures of the Big Hole River, Montana. Submitted to Transactions of the American Fisheries Society.

This report was submitted for publication in Transactions of the American Fisheries Society and was in review when this document (Byorth 1994) was printed. Data from Lohr et al. should be cited only in the context of this document (Byorth 1994).

High Temperature Tolerances of Fluvial Arctic Grayling and Comparisons with Summer River Temperatures of the Big Hole River, Montana

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Abstract

Critical thermal maxima (CTM) and resistance times to high temperatures were determined for juvenile Arctic grayling *Thymallus arcticus* from the fluvial population of the Big Hole River, Montana. Grayling were tested after acclimation to 8.4, 16.0, and 20.0 ^{O}C. Thermal tolerances increased with acclimation temperatures; mean CTM's for the three respective acclimation groups were 26.4, 28.5, and 29.3 ^{O}C, and median resistance time at given test temperature also increased with acclimation. Upper incipient lethal temperature (UILT) was 23.0 ^{O}C for fish acclimated to 8.4 and 16.0 ^{O}C, and 25.0 ^{O}C for those acclimated to 20.0 ^{O}C. Arctic grayling in one group acclimated to 20.0 ^{O}C contracted and were treated for bacterial and parasitic diseases. This group had thermal tolerances more similar to uninfected fish acclimated to lower temperatures of 8.4 and 16.0 ^{O}C than to uninfected fish acclimated to 20.0 ^{O}C. Comparison of CTM and UILT for juvenile Arctic grayling with levels and durations of maximum river temperatures recorded during summer 1992 indicated that resident fish may be occasionally subjected to potentially lethal temperatures in the warmest reaches of the Big Hole River.

Introduction

The Big Hole River in southwestern Montana may contain the only remaining population of exclusively fluvial Arctic grayling Thymallus arcticus in the United States outside of Alaska. Although the species has been introduced to lakes, the Big Hole River population is the only known riverine remnant of a distribution that once included much of the upper Missouri River and its tributaries (Vincent 1962; Kaya 1992). The importance of this remnant population is indicated by evidence that Arctic grayling in Montana are genetically diverged from the more northern populations in Alaska and Canada (Lynch and Vyse 1979; Everett and Allendorf 1985) and that Big Hole River fish appear behaviorally adapted to riverine existence (Kaya 1991). Numbers of grayling in this population have been declining (Kaya 1992), and recent studies have been directed toward monitoring population trends, defining ecological factors affecting their abundance, and exploring means to enhance population size and distribution.

Among the various factors that may be contributing to depression of grayling numbers in the Big Hole River is summer water temperature. The widespread geographic distribution of Arctic grayling in more northern latitudes in Canada, Alaska, and Siberia indicates that those in Montana may be glacial relicts existing in marginal thermal habitats (Vincent 1962). Byorth (1993) recorded a maximum temperature of 27.5 ^{o}C in the river, which exceeds the critical thermal maximum of 26.9 ^{o}C of adult

Arctic grayling from a high-elevation mountain lake in Montana (Feldmeth and Eriksen 1978) and the median tolerance limit (survival of 50% of test fish for 96 h) of about 24.5 °C of juvenile Arctic grayling from an Alaskan population (LaPerriere and Carlson 1973). The tolerance limits determined by these two studies could have been influenced by the relatively cold habitats from which the test fish originated or by their acclimation histories prior to being tested, and information has been lacking on thermal tolerances of fluvial Arctic grayling in Montana.

The objective of this study was to compare thermal tolerances of Arctic grayling from the Big Hole River population with summer temperatures in the river to help evaluate effects of water temperature on this population. We conducted laboratory tests on juvenile fish to determine two aspects of their thermal tolerance: their critical thermal maxima (CTM) and their resistance times to different high temperatures. We considered the CTM to be functionally equivalent to a temperature of instantaneous death because it is "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles and Bogert 1944). Tests of resistance times indicate the survivable durations of exposure to temperatures that are eventually lethal, within a few minutes to 1 week (10,080 min), and the upper incipient lethal temperature (UILT) survivable for indefinitely long periods by 50% of the test population. We compared results of the thermal bioassays with temperatures continually recorded on the Big Hole River during summer 1992.

Methods

Bioassays

Juvenile Arctic grayling used in thermal tolerance tests were F_1 progeny of Big Hole River fish maintained as a reserve brood stock in Axolotl Lake, Madison County, Montana. This brood stock consists of fish taken from the river in 1988 as embryos produced by stripping gametes from freshly captured adults. Gametes were obtained from this brood stock in spring 1992, and the resulting embryos and young were incubated and reared at the Fish Technology Center (U.S. Fish and Wildlife Service) in Bozeman. Newly hatched young were placed into a 0.61-m-diameter tank at 12-14 ^{o}C until they began to feed. Thereafter, the young were reared in circular tanks (1.2 m diameter), with continuous flow of spring water at about 8.4 ^{o}C.

In March 1993, about 80 fish were transferred to each of three 100-L rectangular tanks for acclimation to temperatures of 8.4, 16.0, and 20.0 ^{o}C. These three acclimation levels approximated the average of the median temperature between the daily mean and maximum temperatures of the Big Hole River over three periods - spring and fall (9.5 ^{o}C), early summer (16.4 ^{o}C), and late summer (19.5 ^{o}C) (P. A. Byorth, unpublished data). We selected acclimation temperatures greater than the daily mean values because Hokanson et al. (1977) concluded that, in a fluctuating thermal environment, rainbow trout Oncorhynchus mykiss acclimated to a value between the daily mean and maximum temperatures. Temperature was raised to acclimation levels at the rate of 2-3 ^{o}C per day and maintained within 0.5 ^{o}C with thermostatically controlled immersion heaters. Water was replaced in the tanks at a flow rate of 1.0-1.3 L/min and was kept aerated and circulated with compressed air delivered through submerged airstones. Dissolved oxygen was monitored with a meter (Yellow Springs Instrument Co., Ohio) and remained at 5.0 mg/L or higher in all tanks. An artificial photoperiod of 16:8 h of light:dark was used to simulate summer daylengths. The fish were acclimated for 14 d at 16.0 ^{o}C and 22 d at 20.0 ^{o}C before being tested. They were fed trout pellets during acclimation but were not fed the day before or during thermal tolerance tests. Lengths, weights, and numbers of fish used in tolerance tests are presented in Table 1.

We determined the critical thermal maximum (CTM) of acclimated fish through procedures similar to those described by Hutchison (1961) and Paladino et al. (1980). Individual fish were placed in a 17-L test tank at the acclimation temperature, and temperature was raised 0.4 ^{o}C/min with a 500-W heater. Water in the test tank was kept aerated and vigorously circulated with compressed air released through a submerged airstone. The temperature at which the fish became unable to maintain its equilibrium was recorded as its CTM. Tests were conducted daily from 0800 to 1700 h, until 17-20 fish from each of the three acclimation groups had been tested. Critical thermal maxima were compared among acclimation groups with Kruskal-Wallis tests and Newman-Keuls multiple comparisons (Zar 1984).

Methods for measuring resistance times of fish at different test temperatures and the upper incipient lethal temperatures (UILT) of each acclimation group (the temperature that 50% of the test fish could survive indefinitely) were similar to those described by Brett (1952) and Kaya (1978). Five 100-L test tanks were maintained at water temperatures of 22, 24, 26, 28 and 30 $^{\circ}C$ (± 0.1 $^{\circ}C$). Procedures for maintaining temperature and providing aeration and water circulation were as described for the acclimation tanks. We placed 10 fish from a given acclimation group into each tank and recorded individual times to death, defined as cessation of opercular movement. We monitored the test tanks continuously for the first 4 h, then at intervals not exceeding 30 min for the next 4 h, 2 h for the next 4 h, 3 h for the next 18 h, and 8 h for the remaining time until 10,080 hours (1 week) had elapsed.

Data on thermal resistance times were fitted by least squares regression for each acclimation group, with median resistance time ($\log_{10}$) as the independent variable (for test temperatures that resulted in 50% or greater mortality) and test temperature as the dependent variable. The UILT for each acclimation group was estimated as the midpoint between the lowest temperature producing 50% or greater mortality and the highest temperature producing less than 50% mortality (Edsall and Colby 1970).

During the acclimation period, Arctic grayling in the 20.0 $^{\circ}C$ acclimation group contracted bacterial and parasitic infections, primarily species of Flexibacter, Aeromonas, Pseudomonas, and Ichtyobodo (B. MacConnell, Fish Technology Center, pers. comm.). This diseased group, designated group 20-D, was treated with chloramine-t and formalin (75 ppm) and then used in thermal tolerance tests. Another group of Arctic grayling was taken from the same rearing tank as the original acclimation groups and was acclimated to 20.0 °C for 7 d before being tested. This shorter acclimation period was selected to minimize exposure of fish to the same pathogens. Acclimation to temperatures of 20 °C or higher occurs rapidly, within a few days (Brett 1956; Hutchison 1976). Unless otherwise specified, results from fish acclimated to 20.0 °C refer to this replacement group. Fish acclimated to 8.4 and 16.0 °C were not similarly infected.

Mild gas supersaturation was also observed in the 20.0 °C acclimation tank. Gas content was occasionally measured with a saturometer (Sweeney Aquamatic, Stony Creek, Connecticut). The highest total gas pressure measured was 111.2%, and the highest nitrogen pressure was 114.8%. We observed external emboli in two individuals of the 20-D group and one individual of the replacement 20.0 °C acclimation group. These elevated gas saturation levels were probably produced by the heating of water flowing into the acclimation tanks (G. Kindschi, Fish Technology Center, pers. comm.). Gas saturation levels were considerably lower in the 8.4 and 16.0 °C acclimation tanks and in all test tanks ($\leq$106.1% for both total gas and nitrogen).

Big Hole River Temperatures

Five temperature recording stations were established during 1992 along the section of upper Big Hole River in which grayling are most common (Figure 1). Datapod 212 thermographs (Omnidata International, Inc., Logan, Utah) recorded temperature at 120-min intervals at four stations (1, 3-5) during April-October. Temperature records from a U.S. Geological Survey gauging station (USGS 1992) were used for Station 2. Maximum and mean daily water temperatures and duration (hours) of potentially lethal temperatures (temperatures exceeding the highest estimated UILT) were calculated from the recordings. Maximum daily river temperatures were compared with the mean CTM for each acclimation group, and durations of potentially lethal river temperatures were compared with median resistance times at corresponding test temperatures.

Results

Mean CTM, resistance times to lethal temperatures, and UILT increased with acclimation to higher temperature. Mean CTM's were 26.4, 28.5, and 29.3 ^{o}C for grayling acclimated to 8.4, 16.0, and 20.0 ^{o}C, respectively (Table 2). Differences in CTM's were significant among all acclimation groups ($P<0.01$, Kruskal-Wallis; $P<0.05$, Newman-Keuls multiple comparisons). The mean CTM of the diseased group 20-D was 27.1 ^{o}C, between that of groups acclimated to 8.4 and 16.0 ^{o}C.

Median resistance times were less than or equal to 3 min at 30.0 °C for all acclimation groups but increased with acclimation temperature at test temperatures of 28, 26, and 24 °C (Figure 2). With the exception of the diseased group 20-D, all fish from the three acclimation groups survived at 22.0 °C, and all fish acclimated to 20.0 °C survived at 24 °C. UILT was 23.0 °C for the groups acclimated to 8.4 and 16.0 °C, and 25.0 °C for the group acclimated to 20.0 °C (Table 3). Variances explained by inverse relations between median resistance times and test temperatures were high (R^2 = 0.902-1.000; Table 3).

For acclimation group 20-D, 90% of test fish survived at 22.0 °C, and median resistance times at 26.0 and 28.0 °C were similar to or intermediate between those of the 8.4 and 16.0 °C acclimation groups (Figure 2). At 24.0 °C, the median resistance time of 152 min for group 20-D was less than that of the other acclimation groups - 547 min for the 8.4 °C group, 2593 min for the 16.0 °C group, and >10,080 min (100% survival) for the 20.0 °C group. The estimated UILT of group 20-D (23.0 °C) was similar to that of groups acclimated to lower temperatures of 8.4 and 16.0 °C (Table 3).

Maximum daily temperatures in the Big Hole River during 1992 ranged from 22.0 °C at Station 1 to 27.5 and 26.5 °C at Stations 4 and 5, respectively (Table 4). The two latter temperatures exceeded the mean CTM of 26.4 °C for juvenile Arctic grayling acclimated to 8.4 °C but not for those acclimated to 16.0 or 20.0 °C. Maximum daily temperature exceeded the highest UILT, 25.0 °C for Arctic grayling acclimated to 20.0 °C, for 2 h on 1 d at

Station 3, for 2-6 h on 11 d at Station 4, and for 2-6 h on 3 d at Station 5 (Table 4). Highest mean daily temperatures ranged from 17.8 to 21.4 °C and did not reach or exceed mean CTM or UILT of any acclimation group.

Discussion

The increases in mean CTM, median survival time, and UILT of Big Hole River Arctic grayling produced by higher acclimation temperatures are consistent with thermal responses reported for other fishes (reviewed by Brett 1956; Hutchison 1976). The highest mean CTM of 29.3 °C for this study (at 20.0 °C acclimation) is somewhat higher than those reported by Feldmeth and Eriksen (1978), 26.9 °C for adult and 28.7 °C for age-0 Arctic grayling, respectively, acclimated to only one temperature, 13.0 °C. The difference may be related to origins of fish or acclimation temperature (13.0 vs. 20.0 °C). The mean CTM of 28.5 °C for age-1 juveniles in this study acclimated to 16.0 °C was similar to the mean CTM of 28.7 °C measured by Feldmeth and Eriksen (1978) for age-0 juveniles acclimated to 13 °C.

Maximum daily temperatures of the Big Hole River during 1992 did not appear high enough to produce instantaneous mortality of juvenile Arctic grayling. Maximum daily temperatures did exceed the mean CTM of 26.4 °C for fish acclimated to 8.4 °C but not the mean CTM for fish acclimated to 16.0 or 20.0 °C. Because Arctic grayling in the river would be experiencing warmer temperatures during summer (Table 4) and because acclimation to temperatures above 20 °C occurs rapidly (Brett 1956; Hutchison 1976), their thermal tolerances would probably be those of fish acclimated to higher temperatures. While not high enough to produce instantaneous mortality, the highest temperature of 27.5 °C at station 4 was only about 2 °C below the highest mean CTM (29.3 °C) measured in this study for juvenile Arctic grayling. This relatively small margin of safety would disappear with only slight change in river temperature.

Although not high enough to produce instantaneous mortality of juvenile Arctic grayling, the highest maximum daily river temperatures during 1992 appeared to have been maintained for sufficient durations to be potentially lethal in the warmest reaches. Daily maximum temperatures equaled or exceeded 25.0 °C, the highest UILT estimated for the test fish, at stations 3, 4, and 5 for durations of 2-6 h (120-360 min). Median resistance times for Arctic grayling acclimated to 20.0 °C were 27.5 min at 28.0 °C and 254.5 min at 26.0 °C. Although comparisons between median resistance times and durations of river temperatures were limited by the 120-min intervals of data points recorded by the thermographs, the results indicate that durations of the highest temperature could be potentially lethal to juveniles. This would also apply to larger fish because adult Arctic grayling may have lower thermal tolerances than young fish (Feldmeth and Eriksen 1978).

The UILT's observed in this study, 23.0 and 25.0 °C for 13- to 15-cm fish acclimated to 8.4 to 20.0 °C, were similar to 96-h median tolerance limits of 22.5 to "above 24.5 °C" reported for 5.5- to >20-cm Arctic grayling from interior Alaska acclimated to 4.0 to 8.5 °C (LaPerriere and Carlson 1973). Although the studies differed in sizes of fish, acclimation temperatures, and duration of exposure times (4 d vs. 7 d) employed, the results were similar enough to indicate that Arctic grayling in Montana may not be adapted to tolerate higher temperatures than those from more northern latitudes. This would support a theory that Arctic grayling in Montana represent glacial relicts existing in marginal thermal habitats. Fishes have often been found to exhibit little or no intraspecific variation in thermal tolerances, even among populations widely separated in latitude (Matthews 1986) or isolated in unusual thermal habitats (Brown and Feldmeth 1971; Kaya et al. 1992). Whether Arctic grayling are also evolutionarily conservative in their thermal tolerances warrants further investigation.

Because our highest acclimation temperature was 20.0 °C, the highest UILT that can be produced by acclimation (the ultimate UILT) could be slightly higher than 25.0 °C. Also, fish acclimated to fluctuating temperatures have longer resistance times than those acclimated to a constant temperature equal to the mean of the fluctuations (Otto 1974; Hokanson et al. 1977; Feminella and Matthews 1984). However, the similarity between median survival times of Arctic grayling acclimated at 16.0 and 20.0 °C (Figure 2) indicates that acclimation to temperature above 20.0 °C would have little additional effect on survival times and that their ultimate UILT is near 25.0 °C. Their apparent susceptibility to disease when maintained at 20.0 °C indicates that they may be difficult to acclimate to higher temperatures.

Exposure to pathogens and susceptibility to disease may increase at temperatures that are not directly lethal to fish. Higher incidences of bacterial or parasitic infections have been correlated with warmer water temperatures for other salmonids (Holt et al. 1975; Kaeding 1981; Reeves et al. 1987) and other fishes (Eure and Esch 1974; Esch et al. 1976). High mortality of sockeye salmon O. nerka has been attributed to bacterial infections exacerbated by elevated water temperatures in the Columbia River (Fish 1948). In this study, Arctic grayling that had been infected by and treated for bacterial and parasitic infections had lower CTM's and shorter median resistance times than others acclimated to the same temperature (20.0 °C). Because we did not subject healthy controls to the same disease treatments, we do not know whether the lower thermal tolerances resulted from the diseases or the treatments. Although inconclusive, these results raise the possibility that warmer temperatures in sections of the Big Hole River may result in increased susceptibility of Arctic grayling to bacterial and parasitic infections, and that such increased susceptibility to infections could indirectly lead to mortalities under warmer summer temperatures.

Although no thermal fish kills have been observed in the Big Hole River, the warmest reaches of the upper river (Figure 1) contain lower densities of Arctic grayling (Byorth 1993).

Behavioral avoidance of these temperatures, or numerous other factors, may be influencing this pattern of distribution, including other aspects of habitat quality, angling pressure, and competition from non-native fishes. Temperature is only one factor affecting fish populations, and given that Arctic grayling have declined throughout this river and disappeared from all other streams of the upper Missouri River drainage, these other factors must be important. These comparisons, between thermal tolerances of juvenile Arctic grayling and river temperatures during 1992, indicate possible thermal problems for resident Arctic grayling. Post-glacial climatic changes and additional warming of stream temperatures from human activities may be contributing toward placing this remnant population at risk of extirpation. Future work on possible thermally related problems affecting this population should include determining thermal tolerances of other sizes and ages of fish, evaluating effects of temperature on interspecific interactions between Arctic grayling and the non-native salmonids established in the river, and evaluating susceptibility to bacterial and parasitic infections.

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Footnotes

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Table 1. Mean length (SD), weight (SD), and number of Arctic grayling from each acclimation group used in tests of critical thermal maximum and thermal resistance time. Groups correspond to acclimation temperatures; group 20-D was composed of diseased fish acclimated to 20.0 °C.

Acclimation group	Length (mm)	Weight (g)	N
	Critical thermal maximum		
8.4	139(7)	20.4(3.9)	20
16.0	146(7)	23.6(3.4)	18
20.0	127(5)	14.1(1.7)	17
20-D	135(6)	16.9(3.1)	20
	Thermal resistance time		
8.4	132(9)	17.3(3.8)	50
16.0	139(8)	19.9(4.2)	50
20.0	128(6)	14.8(2.5)	50
20-D	133(9)	16.6(4.4)	50

Table 2. Mean, standard deviation, and range for the critical thermal maximum and number of Arctic grayling from each acclimation group. Groups correspond to acclimation temperatures; group 20-D was composed of diseased fish acclimated to 20.0 °C.

Acclimation		Critical thermal maximum (°C)		
group	N	Mean	SD	Range
8.4	20	26.4	0.8	24.6-27.5
16.0	18	28.5	0.4	27.7-29.4
20.0	17	29.3	0.3	28.6-29.7
20-D	20	27.1	0.7	26.3-28.7

Table 3. Slope (SE), intercept (SE), and coefficient of determination (R^2) of thermal resistance times and estimated upper incipient lethal temperature (UILT) for each acclimation group of Arctic grayling. Regression equations used $\log_{10}$ median resistance times and test temperatures as independent and dependent variables, respectively. Groups correspond to acclimation temperatures; group 20-D was composed of diseased fish acclimated to 20.0 ^{o}C.

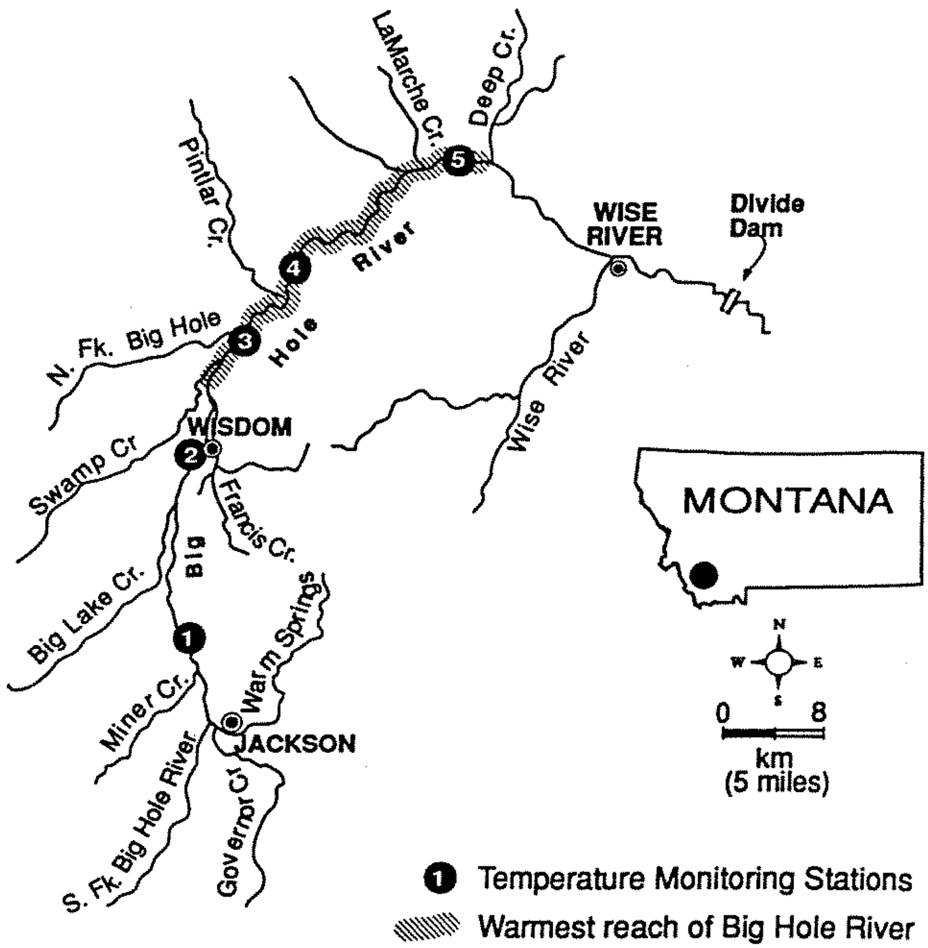
Acclimation group	Slope	Intercept	R^2	UILT (^{o}C)
8.4	-2.31(0.54)	29.90(0.84)	0.902	23.0
16.0	-1.93(0.16)	30.35(0.34)	0.986	23.0
20.0	2.07(<0.01)	30.99(<0.01)	1.000	25.0
20-D	-3.28(0.43)	31.03(0.60)	0.967	23.0

Table 4. Maximum daily water temperature (T_{max}), highest mean daily temperature (Maximum T_{mean}), and number of days and hours per day that maximum daily temperature equaled or exceeded 25.0 ^{o}C during 1992 at five thermograph stations in the Big Hole River, Montana. Station locations are presented in Figure 1.

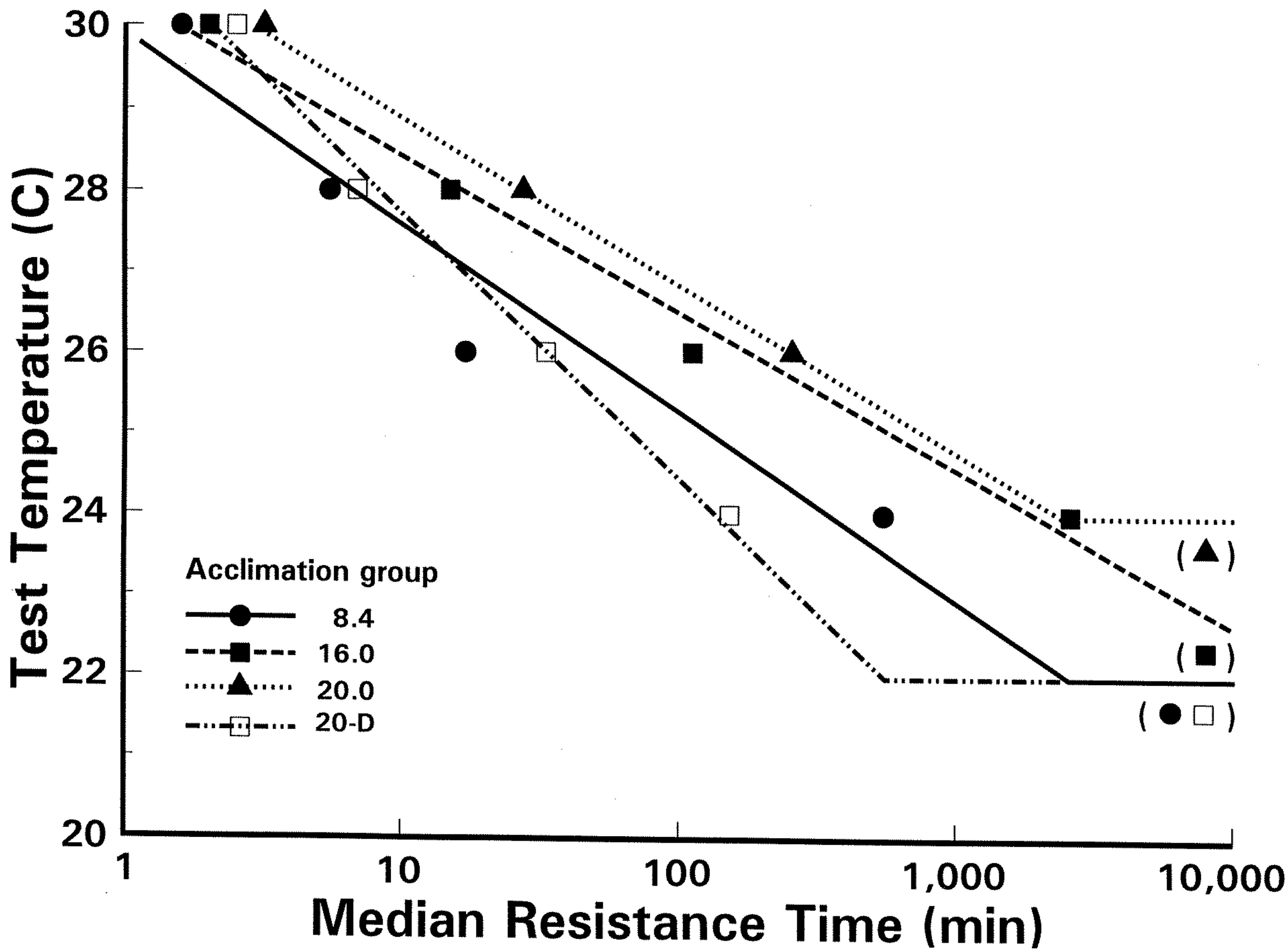
Station	T_{max} (^{o}C)	Maximum T_{mean} (^{o}C)	Days T_{max} $\geq$ 25.0 ^{o}C	Hours per day T_{max} $\geq$ 25.0 ^{o}C
1	22.0	17.8	0	0
2	24.4	19.1	0	0
3	25.0	20.6	1	2
4	27.5	20.3	11	2,2,4,4,4, 4,4,4,4,6,6
5	26.5	21.4	3	2,2,6

Figure 1. Map of the upper Big Hole River, Montana. Thermograph stations are designated by number; the warmest reach is shaded. Arctic grayling are most common from about 6 km above Wisdom to the Divide Dam and in the lowest reaches of the tributaries in this section.

Figure 2. Median resistance times of Arctic grayling by acclimation group. Groups correspond to acclimation temperature; group 20-D was composed of diseased fish acclimated to 20.0 ^{o}C. Symbols in parentheses indicate temperatures at which more than 50% of the test fish survived.



LaMarche Cr.
Deep Cr.
Pintlar Cr.
WISE RIVER
Divide Dam
River
N. Fk. Big Hole
Hole
WISDOM
Swamp Cr
Francis Cr.
Wise River
MONTANA
Big Lake Cr.
Big
Warm Springs
Miner Cr.
JACKSON
S. Fk. Big Hole River
Governor Cr.
N
W
E
S
0
8
km
(5 miles)
Temperature Monitoring Stations
Warmest reach of Big Hole River


Test Temperature (C)
30
28
26
24
22
20
1
10
100
1,000
10,000
Median Resistance Time (min)
Acclimation group
8.4
16.0
20.0
20-D