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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 maximum (CTM) and resistance time to high temperature 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°C. Thermal tolerances increased with acclimation temperatures; mean CTM was 26.4°C for the 8.4°C acclimation group, 28.5°C for the 16.0°C group, and 29.3°C for the 20.0°C group; median resistance time at given test temperature also increased with acclimation. The upper incipient lethal temperature (UILT) was 23.0°C for fish acclimated to 8.4°C and 16.0°C, and was 25.0°C for those acclimated to 20.0°C, temperatures that were similar to the median tolerance limits of Arctic grayling in Alaska. Comparisons of mean CTM and UILT for juvenile Arctic grayling with levels and durations of maximum river temperatures recorded during summers 1992–1994 indicated that resident fish may occasionally be subjected to potentially lethal temperatures in the warmest reaches of the Big Hole River.

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 the species' historical distribution that once included much of the upper Missouri River and its tributaries (Vincent 1962; Kaya 1992). The importance of this 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 to be behaviorally adapted to riverine existence (Kaya 1991). Moreover, attempts to reintroduce Arctic grayling into streams have failed, presumably because fish of lacustrine origin were used and because other factors that contributed to their extirpation are still present (Kaya 1992). The number of Arctic grayling in the Big Hole River has been declining (Kaya 1992), and recent studies have been directed toward monitoring population trends, defining ecological factors affecting abundance, and exploring means to enhance population size and distribution.

Among the various factors that may be contributing to depression of Arctic 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). Thermographs at five stations in the Big Hole River during summers of 1992–1994 recorded a maximum water tempera-

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ture of 27.5°C at one station in July 1992 (unpublished data). This temperature exceeded the critical thermal maximum (CTM) of 26.9°C for 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 for juvenile Arctic grayling from an Alaskan population (LaPerriere and Carlson 1973). Moreover, a fish kill that included Arctic grayling and seven other species occurred during the period of warmest water temperatures in a reach of the Big Hole River in July 1994.

The objective of this study was to examine the occurrence of temperatures potentially lethal to Arctic grayling in the Big Hole River population. We determined thermal tolerances of juvenile fish to elevated temperatures in laboratory tests, and then compared tolerance limits with thermograph records of the river during summers of 1992–1994.

Methods

We conducted laboratory tests on juvenile fish to determine two aspects of their thermal tolerance: CTM and resistance time to different high temperatures. We considered the CTM to be functionally equivalent to a temperature causing 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—from within a few minutes to 1 week (10,080 min)—of exposure to temperatures that are eventually lethal and also indicate the upper incipient lethal temperature (UILT). The UILT is the temperature that is survivable indefinitely (for periods longer than 1 week) by 50% of the test population.

Thermal Tolerance Tests

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

In March 1993, about 80 fish were transferred to each of three 100-L rectangular tanks. Fish in one tank were held at 8.4°C, and those in the other two were acclimated to 16.0°C and 20.0°C. These three acclimation levels approximated our estimates of average daily acclimation temperatures experienced by fish in the Big Hole River during three periods in 1992: spring and fall (9.5°C), early summer (16.4°C), and late summer (19.5°C). We assumed that on a given day, the mean acclimation temperature for fish in the river was represented by the median between the mean and maximum temperatures for that date. These daily medians were obtained from unpublished thermograph data for 1992 and were averaged for these three periods. The estimates of daily acclimation temperatures were based on the conclusion by Hokanson et al. (1977) that, in a fluctuating thermal environment, rainbow trout *Oncorhynchus mykiss* became acclimated to a level between daily mean and maximum temperatures.

We acclimated fish in one of the holding tanks to 16.0°C by raising the temperature from 8.4°C to 16.0°C during 3 d and maintaining water temperature at 16.0°C for at least 14 d before using fish in tests. We similarly acclimated fish to 20.0°C, but they contracted bacterial and parasitic infections within the 14-d period at 20.0°C and are not included in the results. In April we acclimated a second group of fish by raising water temperature from 8.4°C to 20.0°C during 4 d and maintaining the temperature at 20.0°C for at least 7 d before using fish in tests. We thought that maintaining fish at 20.0°C for only 7 d instead of 14 d would minimize their exposure to pathogens. Moreover, because fish acclimate rapidly to high temperatures (Brett 1956; Heath 1963; Holland et al. 1974; Hutchison 1976), we assumed that they would be fully acclimated within a week. No pathogens were found in fish from either the second group acclimated to 20.0°C or in those acclimated to 8.4°C and 16.0°C.

During the acclimation periods, water temperature was maintained within 0.5°C of target by use of thermostatically controlled immersion heaters. Water was replaced in the tanks at a flow rate of 1.0–1.3 L/min and was aerated and circulated with compressed air delivered through submerged airstones. Dissolved oxygen was monitored with a Yellow Springs Instruments Corp. dissolved oxygen meter and was at least 5.0 mg/L in all tanks. A photoperiod of 16 h light:8 h dark was used to simulate summer day length. All fish were fed trout pellets during acclimation but were not fed the day before or during thermal tolerance tests.

We determined the CTM of acclimated fish by using procedures similar to those described by Hutchison (1961) and Paladino et al. (1980). An individual fish was placed in a 17-L test tank at the fish's acclimation temperature, and temperature was raised 0.4°C/min with a 500-W heater. Although dissolved oxygen measurements were not recorded in the test tank, water was aerated and vigorously circulated with compressed air released through a submerged airstone to prevent thermal stratification. The temperature at which a fish became unable to maintain equilibrium was recorded as its CTM. Tests were conducted daily from 0800 to 1700 hours until 17–20 fish from each of the three acclimation groups had been tested (mean total length: 137.5 mm, SD = 10.1; mean weight: 19.5 g, SD = 5.1; all fish combined, after testing). 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 UILT of each acclimation group 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°C (±0.1°C). Procedures for maintaining temperature and providing aeration and water circulation in each test tank were as described for the acclimation tanks. Periodic measurements during tests showed dissolved oxygen was greater than 5.0 mg/L in all test tanks. We placed 10 fish from a given acclimation group (mean total length: 133.0 mm, SD = 8.7; mean weight: 17.3 g, SD = 4.1; all fish combined, after testing) into each tank and recorded individual times to death, which was defined as cessation of opercular movement. We monitored the test tanks continuously for the first 4 h, then at least every 30 min for the next 4 h, every 2 h for the next 4 h, every 3 h for the next 18 h, and every 8 h for the remaining time until 10,080 min (1 week) had elapsed.

Thermal resistance times for test temperatures that resulted in 50% mortality or higher were fitted by least-squares regression for each acclimation group; test temperature was the independent variable and median resistance time ($\log_{10}$) was the dependent variable. The UILT for each acclimation group was estimated as the midpoint between the lowest test temperature that resulted in at least 50% mortality and the next lower test temperature producing less than 50% mortality.

We observed mild gas supersaturation in the 20.0°C tank during acclimation. Gas content was occasionally measured with a saturometer (Sweeney Aquamatic, Stony Creek, Connecticut). For three measurements in this tank, mean total gas pressure was 106.4% of saturation (range, 103.3–111.2%), and mean nitrogen pressure was 113.3% (range, 111.9–114.8%). However, we observed external emboli in only one fish. Elevated dissolved gas saturations were produced by the heating of gas-saturated water flowing into this acclimation tank. Single measurements of dissolved gas saturation were lower in both the 8.4°C and 16.0°C acclimation tanks and in all test tanks used for determining thermal resistance times (≤106.1% for both total gas and nitrogen).

Big Hole River Temperatures

Five temperature recording stations were established during 1992–1994 along the section of upper Big Hole River where Arctic grayling are most common (Figure 1). Thermographs (Datapod 212, Omnidata International, Inc., Logan, Utah) recorded temperature at 120-min intervals at stations 1, 3, 4, and 5 during April–October each year. At station 2, temperature records from a U.S. Geological Survey gauging station (06024450; USGS 1993–1995) provided hourly measurements. Maximum and mean daily water temperatures and duration (h) of potentially lethal temperatures that exceeded the highest estimated UILT were calculated from the records. Maximum daily river temperatures were compared with the mean CTM for each acclimation group of Arctic grayling, and durations of potentially lethal river temperatures were compared with median resistance times at corresponding test temperatures.

Results

Mean CTM, median resistance time to lethal temperature, and UILT increased with acclimation to higher temperatures. Mean CTMs were 26.4°C for the 8.4°C acclimation group, 28.5°C for the 16.0°C group, and 29.3°C for the 20.0°C group (Table 1). Moreover, the median CTM of each acclimation group differed no more than 0.2°C from each mean. Differences in CTMs were significant among all acclimation groups ($P < 0.01$, Kruskal–Wallis; $P < 0.05$, Newman–Keuls multiple comparisons).

During tests of thermal resistance times, all fish from the three acclimation groups survived for one

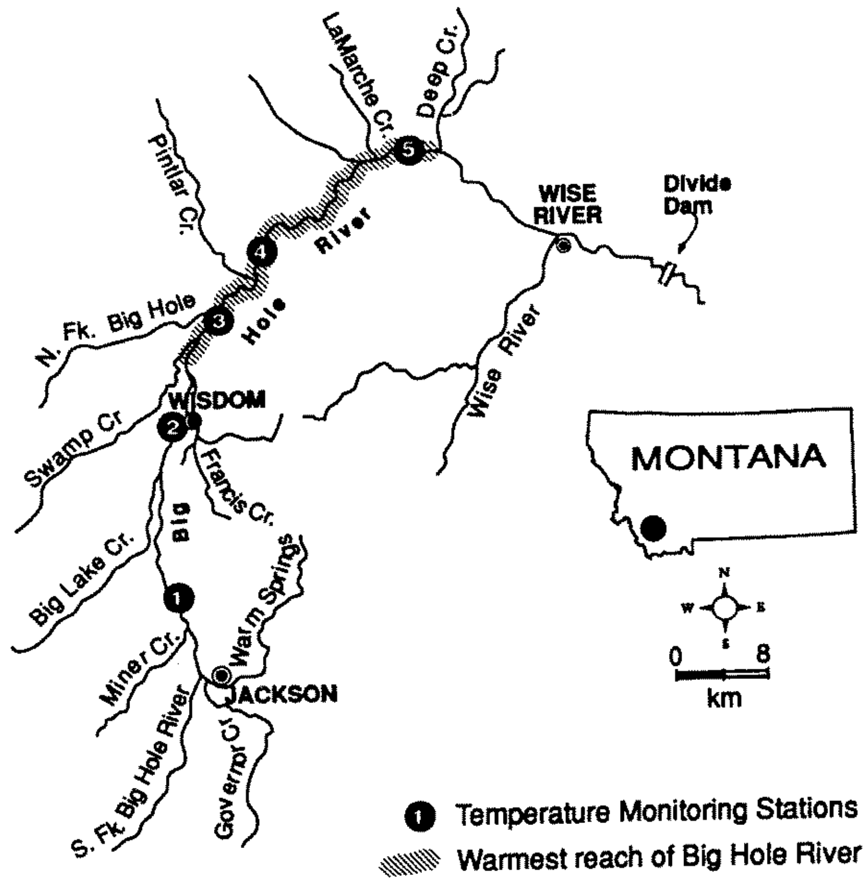

FIGURE 1.—Map of the upper Big Hole River, Montana. Thermograph stations are designated by number; the warmest reach is shaded. Arctic grayling are most common from about 6 km above Wisdom to the Divide Dam and in the lowest reaches of the tributaries in this section.

week at a test temperature of 22.0°C, and all fish acclimated to 20.0°C survived for one week at 24.0°C (Table 2). Because 50% or fewer of the fish acclimated to 8.4°C and 16.0°C survived at test temperatures of 24.0°C or higher, the UILT was 23.0°C for these groups. However, for fish acclimated to 20.0°C, none survived at 26.0°C or higher so the UILT was 25.0°C. For each acclimation group, linear regressions between median resistance times and the three or four test temperatures that resulted in 50% or higher mortality were significant ($P \leq 0.050$; $r^2 = 0.902$–1.000).

Maximum daily water temperatures in the Big Hole River during 1992–1994 ranged from 20.0°C at station 5 in 1993 to 27.5°C at station 4 in 1992 (Table 3). Temperatures in 1993 were lower than in 1992 and 1994 and did not exceed the mean CTM or UILT of juvenile Arctic grayling at the three acclimation temperatures we tested. However, maximum daily temperature equaled or exceeded the highest UILT (25.0°C for Arctic grayling acclimated to 20.0°C) during 1–11 d at three stations in 1992 and 1994. On each of these days, temperatures were 25.0°C or higher for up to 6 h. The highest mean daily temperatures ranged from 16.7°C to 21.8°C and did not equal or exceed the mean CTM or UILT of any acclimation group.

Discussion

Maximum daily temperatures of the Big Hole River during 1992–1994 did not appear to be acutely lethal for juvenile Arctic grayling. River temperatures exceeded 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°C or 20.0°C. Because Arctic grayling in the river would be experiencing warmer temperatures during summer, and because their acclimation to temperatures above 20°C oc-

TABLE 1.—Critical thermal maximum of Arctic grayling acclimated to three different temperatures.

Acclimation temperature (°C)	*N*	Critical thermal maximum (°C)			
		Mean	SD	Median	Range
8.4	20	26.4	0.8	26.6	24.6–27.5
16.0	18	28.5	0.4	28.4	27.7–29.4
20.0	17	29.3	0.3	29.4	28.6–29.7

TABLE 3.—Maximum daily water temperature (T_{max}), highest mean daily temperature (maximum T_{mean}), and number of days and hours per day when maximum daily temperature equaled or exceeded 25.0°C in 1992–1994 at five thermograph stations in the Big Hole River, Montana. Station locations are presented in Figure 1.

Year and station	T_{max} (°C)	Maximum T_{mean} (°C)	$T_{max} \geq 25.0$°C	
			Days	Hours/day
1992				
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
1993				
1	20.5	16.7	0	0
2	21.7	18.1	0	0
3	21.5	18.7	0	0
4	21.0	18.3	0	0
5	20.0	17.7	0	0
1994				
1	24.0	18.8	0	0
2	25.4	20.8	4	1,1,2,3
3	25.0	21.3	2	2,4
4	26.5	21.8	8	2,2,2,2,2,6,6,6
5	24.5	18.9	0	0

curs rapidly (Brett 1956; Hutchison 1976), we assumed their thermal tolerances would be those of fish acclimated to higher temperatures. Although not acutely lethal, the highest temperature of 27.5°C at station 4 in 1992 was only about 2°C below the highest mean CTM (29.3°C) measured in our tests. This relatively small margin of safety would disappear with only slight change in river temperature.

The highest maximum daily river temperatures during 1992 and 1994 seem to have been maintained for sufficient durations to be potentially lethal in the warmest reaches. These temperatures equaled or exceeded 25.0°C, the highest UILT estimated for the test fish, at stations 3, 4, and 5 for 2–6 h in 1992, and at stations 2, 3, and 4 for 1–6 h in 1994. Median resistance times for Arctic grayling acclimated to 20.0°C were 27.5 min at 28.0°C and 254.5 min (4.2 h) at 26.0°C. Although comparisons between median resistance times and durations of river temperatures were limited by the 2-h interval between recordings by four of the five thermographs, the results indicate that durations of the highest temperature could be lethal to juveniles and perhaps more so to adults, which may have lower thermal tolerances than young fish (Feldmeth and Eriksen 1978).

The UILTs observed in this study—23.0°C and 25.0°C, for fish about 14 cm (total length) and acclimated at 8.4, 16.0, and 20.0°C—were similar to 96-h median tolerance limits of 22.5°C to "above 24.5°C" reported for Arctic grayling (total length 5.5 cm to >20 cm) from interior Alaska that were acclimated to 4.0–8.5°C (LaPerriere and Carlson 1973). Although the two studies differed in sizes of fish, acclimation temperatures, and durations of exposure time (4 versus 7 d), the results from both suggest that Arctic grayling in Montana may not be adapted to tolerate higher temperatures than fish in more northern latitudes can tolerate. This would support a theory that Arctic grayling in Montana represent glacial relicts existing in marginal thermal habitats. Although some researchers have found intraspecific differences in thermal tolerances among populations of other species (Holland et al. 1974; Feminella and Matthews 1984), others have found little or no intraspecific variation among populations widely separated in latitude (Matthews 1986) or isolated in unusual thermal habitats (Brown and Feldmeth 1971; Kaya et al. 1992).

TABLE 2.—Median thermal resistance time and upper incipient lethal temperature (UILT) of Arctic grayling acclimated to three different temperatures and held at various high temperatures for up to one week.

Acclimation temperature (°C)	Median thermal resistance time (min) at					UILT (°C)
	22.0°C	24.0°C	26.0°C	28.0°C	30.0°C	
8.4	>10,080[a]	547[b]	17	5.5	2	23.0
16.0	>10,080[a]	2,593[c]	111.5	15	2	23.0
20.0	>10,080[a]	>10,080[a]	254.5	27.5	3	25.0

[a] All fish survived.
[b] One fish survived.
[c] Two fish survived.

Because our highest acclimation temperature was 20.0°C, and test temperatures for thermal tolerance times were at 2.0°C intervals, the highest UILT that could be produced by acclimation (the ultimate UILT) would be slightly higher than 25.0°C. Furthermore, fish acclimated to fluctuating temperature, as occurs in nature, have longer resistance times than fish acclimated to a constant temperature equal to the mean of the fluctuations (Otto 1974; Hokanson et al. 1977; Feminella and Matthews 1984). However, the relative similarity between median survival times of Arctic grayling acclimated at 16.0°C and 20.0°C 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.

The July 1994 fish kill in the Big Hole River occurred during the warmest months in a reach that contains lower densities of Arctic grayling than other areas of the upper river (unpublished data). Behavioral avoidance of these temperatures may be influencing this pattern of distribution, as may other factors, including other aspects of habitat quality, angling pressure, and competition from nonnative fishes. Temperature is only one factor affecting fish populations, and given that Arctic grayling have declined throughout this river and have disappeared from all other streams of the upper Missouri River drainage, these other factors must be important. The comparisons between thermal tolerances of juvenile Arctic grayling and summer river temperatures indicate possible thermal problems for resident Arctic grayling but do not address spatial and temporal variation of temperature in the river. However, we infer that potential postglacial climatic changes and additional warming of stream temperatures arising from human activities may be helping to place this remnant population at risk of extirpation. Future work on possible thermal-related problems affecting this population should be field-oriented and should include assessing diurnal and seasonal behavioral responses of Arctic grayling to the thermal environment of the river; determining interactions among temperature, growth, and survival; evaluating effects of temperature on interspecific interactions between Arctic grayling and the nonnative salmonids established in the river; and evaluating susceptibility of fish to pathogens at elevated temperatures.

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