Environmental Toxicology and Chemistry, Vol. 20, No. 10, pp. 2342–2352, 2001
Printed in the USA
0730-7268/01 $9.00 + .00

NATURALIZED SALMONID POPULATIONS OCCUR IN THE PRESENCE OF ELEVATED TRACE ELEMENT CONCENTRATIONS AND TEMPERATURES IN THE FIREHOLE RIVER, YELLOWSTONE NATIONAL PARK, WYOMING, USA

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(*Received* 11 *July* 2000; *Accepted* 1 *March* 2001)

Abstract—We investigated the effects of geothermally influenced waters on the distribution of rainbow trout, *Oncorhynchus mykiss*, and brown trout, *Salmo trutta*, in the Firehole River and its tributaries in Yellowstone National Park (WY, USA) from June 1997 to June 1998. Geothermal features in the Firehole River basin elevate mineral content and temperature in portions of the river and its tributaries. We found concentrations of boron and arsenic to be elevated in geothermally influenced areas compared with upstream sites. Boron concentrations occasionally exceeded 1,000 μg/L, a proposed limit for the protection of aquatic organisms. Arsenic concentrations occasionally exceeded 190 μg/L, the chronic ambient water quality criterion. Temperatures in geothermally influenced sites ranged up to 30°C and were consistently 5 to 10°C higher than upstream sites unaffected by geothermal inputs. Rainbow trout occurred at sites with elevated concentrations of boron, arsenic, and other trace elements and elevated water temperatures. Rainbow trout inhabited and spawned at sites with the most elevated trace element concentrations and temperatures; however, brown trout were absent from these sites. Water temperature may be the major factor determining brown trout distributions, but we cannot exclude the possibility that brown trout are more sensitive than rainbow trout to boron, arsenic, or other trace elements. Further investigations are needed to determine species-specific tolerances of boron, arsenic, and other trace elements among salmonids.

Keywords—Rainbow trout Brown trout Boron Arsenic Yellowstone

INTRODUCTION

The Firehole River in Yellowstone National Park (WY, USA) provides a unique opportunity to investigate the effects of elevated concentrations of boron, arsenic, and several other trace elements on salmonids in a natural ecosystem. Although the Firehole River has been used as a model for assessing the long-term ecological effects of thermal pollution [1,2], the chemical gradient increases similarly with the more obvious thermal gradient. Concentrations of major cations and anions in the river increase with progression downstream in the Firehole River. Records [3] indicate that boron concentrations increase sevenfold between Kepler Cascades (WY, USA) and its confluence with the Gibbon River (Fig. 1). Boron concentrations in the lower reaches of the Firehole River have been observed up to 1,000 μg/L [3], a concentration proposed as the maximum acceptable concentration of boron in surface waters for the protection of aquatic organisms based on the sensitivity of rainbow trout, *Oncorhynchus mykiss* [4,5]. However, no previous studies have evaluated the relations of boron, arsenic, or other contaminants to the ecology and distribution of trout in the Firehole River.

We were interested in the relationship between elevated boron concentrations and the distributions of salmonids in the Firehole River because of current regulatory concerns. It is important to assess accurately the potential effects of boron on aquatic organisms because of its numerous uses in residential, agricultural, and industrial applications. For example, sodium perborate is a bleaching agent that can constitute up to 15% of detergents by weight [6]. Common agricultural and residential uses result in direct discharge of boron into surface waters [7–9].

Although the U.S. Environmental Protection Agency has not yet established an aquatic life water quality criterion for boron, both the World Health Organization and the European Union are currently reviewing their regulatory criteria. The European Union has recommended a surface water quality limit for boron of 1,000 μg/L [6], based primarily on results of laboratory toxicity studies with rainbow trout. However, regulatory confidence would be increased if field studies could validate that aquatic biota can persist at concentrations greater than or equal to 1,000 μg/L.

Our purpose was to determine how geothermally influenced waters affect the spatial and temporal variation in water chemistry and distributions of rainbow trout and brown trout, *Salmo trutta*, in the Firehole River drainage. We determined spatial and temporal variability in water chemistry over a one-year period. Rainbow trout and brown trout densities were determined by life stages during the spring, summer, and fall, and spawning sites of rainbow trout and brown trout were located. From these results, we evaluated the influence of water chemistry and temperature on the distribution, densities, and spawning locations of these species in the Firehole River basin.

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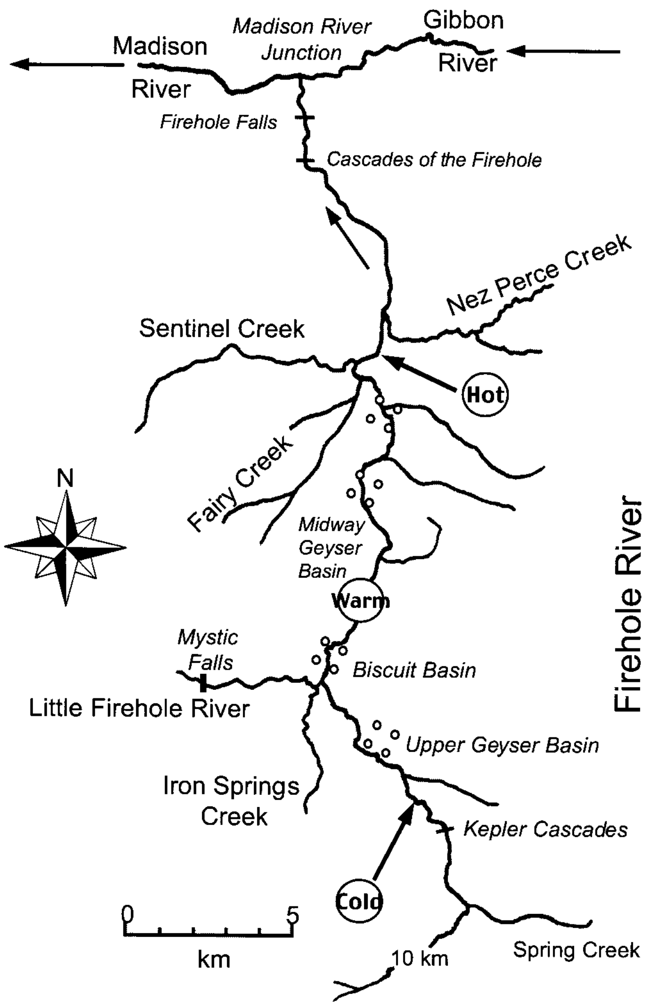

Fig. 1. Firehole River drainage, Yellowstone National Park (WY, USA). Study sites for water quality sampling and trout population surveys in the mainstem of the Firehole River were identified as the cold, warm, and hot sites. Groups of small circles indicate basins where geothermal features were concentrated.

METHODS

Study area and sampling sites

The Firehole River is completely within the boundaries of Yellowstone National Park and joins with the Gibbon River to form the Madison River, becoming one of the headwater rivers of the Missouri River, USA (Fig. 1). The Firehole River ranges from 2,070 to 2,500 m in elevation, is 44 km long, has a mean channel slope of 1.1%, and drains 720 km^2 [10]. Mean discharge is 8 m^3/s, and peak runoff increases to 21 m^3/s during May and June [10,11].

Three major geyser basins increase mineral content and water temperature in the Firehole River. Geothermal waters that enter the Firehole River are chemically enriched by leachate from the bedrock and magmatic gases [12–14], and these geothermally influenced waters can constitute up to 40% of the total Firehole River discharge during periods outside of spring run-off [12]. On an annual basis, the geothermally influenced waters elevate trace element concentrations, including arsenic, boron, and fluoride [3,14], and increase water temperature in the warmest sections of the Firehole River by 11°C above normal [15–17].

The Firehole River was reported to be fishless before salmonids were established by stocking beginning in 1889 [18,19]. The drainage has been stocked with rainbow trout; brown trout; brook trout, *Salvelinus fontinalis*; and cutthroat trout, *O. clarki* [20]. Trout in the Firehole River are isolated from other species and populations that exist downstream in the Madison River drainage by natural barriers (Firehole Falls and Cascades of the Firehole) that prevent upstream migration (Fig. 1). Stocking ceased in 1955, but salmonids in the Firehole River became self-sustaining. Some cutthroat trout populations persist in headwater areas of several tributaries above fish barriers. However, only brown trout and rainbow trout occur in the mainstem and associated tributaries [21]. The river has become a renowned trout fishery.

Eight study sites were established in the Firehole River, between Kepler Cascades and Firehole Falls, with three sites on the mainstem and five on tributaries (Fig. 1). The three mainstem sites represented a gradient of the influence of geothermally influenced waters on water quality and were termed cold, warm, and hot. All tributaries to the Firehole River in this segment were surveyed and tributaries with physical barriers that prevented access by trout from the mainstem of the river were not studied. Tributary sites were Iron Springs Creek, Little Firehole River, Fairy Creek, Sentinel Creek, and Nez Perce Creek.

Water chemistry

Water samples were collected for chemical analyses at each site at least monthly from June 1997 through June 1998, with the exception of the cold site, Sentinel Creek, and Nez Perce Creek (WY, USA), which were only sampled for five months during the summer and fall. Specific conductivity was measured with a YSI Model 85 meter (Yellow Spring Instruments, Yellow Springs, OH, USA). Measurements of pH were made with an Orion 290A pH meter (Orion Research, Boston, MA, USA) or a HACH EC30 meter (Hach, Lakewood, CO, USA). Alkalinity was determined titrimetrically [22]. Samples for determination of ammonia concentrations were collected in acid-washed polyethylene conical tubes and frozen before total ammonia concentrations were determined by colorimetry [23]. Concentrations of un-ionized ammonia (NH_3) were calculated from total ammonia concentrations, pH, and temperature [24]. Samples for total organic carbon (TOC) and dissolved organic carbon (DOC) analyses were collected in level-II clean amber glass bottles and preserved with hydrochloric acid. Water samples for DOC analysis were passed through a 0.7-μm glass-fiber filter before preservation. The TOC and DOC analyses were conducted with a Shimadzu TOC5000A Carbon Analyzer (Shimadzu, Columbia, MD, USA), with a detection limit of 240 μg C/L. Water temperature was measured hourly at each study site using a submersible temperature data logger (Optic Stowaway® Temperature Logger, Onset Computer, Pocasset, MA, USA).

Samples for analysis of total and dissolved cations and trace elements were collected in I-Chem® bottles (Nalge Nunc, Rochester, NY, USA) and preserved with concentrated Ultrex® nitric acid (J.T. Baker, Phillipsburg, NJ, USA). Water for measurement of dissolved concentrations was passed through a 0.45-μm Nuclepore® cellulose-fiber filter (Nuclepore Track-Etch Membrane, Corning Separations, Cambridge, MA, USA). A Perkin-Elmer Sciex Elan 6000 inductively coupled plasma mass spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a discrete dynode detector (ETP Electron Multiplier, Australia) was used to measure concentrations of As, B, Ca, Cr, Mg, Na, and Se in all samples. These elements appeared to be the major trace metals of concern in preliminary

Table 1. Representative normalities of cations and anions at study sites in the Firehole River (WY, USA) and tributaries

Site	Cations (μEq/L)				Anions (μEq/L)			
	Na^+	Ca^{2+}	K^+	Mg^{2+}	HCO_3^-	Cl^-	SO_4^{2-}	F^-
July 14, 1997								
Little Firehole River	389	194	70	35	1,060	73	85	135
Warm site	1,233	166	102	33	880	360	69	282
Iron Springs Creek	1,619	193	138	35	460	663	78	297
Fairy Creek	4,090	205	146	35	2,080	1,484	150	516
Hot site	2,462	189	152	29	1,160	912	113	294
November 24, 1997								
Little Firehole River	565	263	82	37	500	117	144	166
Warm site	1,917	191	132	31	1,220	895	110	272
Iron Springs Creek	1,558	187	132	34	1,000	652	85	258
Fairy Creek	4,679	195	161	34	2,220	2,195	181	537
Hot site	3,558	248	184	29	1,860	1,687	163	383
February 4, 1998								
Little Firehole River	610	284	87	41	680	129	138	173
Warm site	1,850	200	195	33	1,140	1,047	126	348
Iron Springs Creek	1,799	211	159	36	1,140	931	85	299
Fairy Creek	5,416	196	240	39	2,680	2,702	198	616
Hot site	3,922	263	243	30	2,160	2,050	172	403
April 28, 1998								
Little Firehole River	575	264	84	44	620	118	119	142
Warm site	1,975	208	140	37	1,100	894	108	253
Iron Springs Creek	1,901	196	150	36	1,220	812	98	295
Fairy Creek	3,942	169	164	41	1,240	1,862	146	484
Hot site	3,571	231	205	31	1,920	1,907	173	389

inductively coupled plasma mass spectrometer scans of Firehole River water samples [25].

Arsenic was speciated into As(III) and As(V). The concentrations of As(V), in the form of arsenate, were determined by colorimetry [26]. The concentrations of As(III) were determined by difference of the total arsenic and As(V) concentrations from the two separate analytical procedures.

Additionally, samples were collected to determine concentrations of potassium (K^+), sulfate (SO_4^{2-}), nitrate (NO_3^-), chloride (Cl^-), and fluoride (F^-). Concentrations of K^+ were determined by flame atomic absorption spectrophotometry (Perkin-Elmer Model 372), and concentrations of the anions were determined by ion chromatography (Dionex Model 2110i, Sunnyvale, CA, USA).

Salmonids

Estimates of salmonid density and species distribution were conducted at each study site once every two weeks from July 1997 through November 1997 concurrent with water quality sampling, as described by Goldstein [25]. Fish densities were estimated in a 200-m reach at each site using snorkeling methods. Fish were counted by species and the length classes: young of year (<10 cm total length), juvenile (10–30 cm total length), and adult (>30 cm total length). Electrofishing was not used because of impacts on fish from frequent resampling in the same reaches. Additionally, portions of the Firehole River, Little Firehole River, Fairy Creek, and Nez Perce Creek were surveyed for spawning salmonids from November 1997 through February 1998. During each survey, redds (excavations created by spawning trout where eggs are deposited) were counted and their locations were recorded. Fry were collected from March through May 1998 in the areas where spawning was observed, and they were identified as rainbow trout or brown trout using meristic and morphometric measurements.

Linear regression analysis was used to determine if relationships existed between water chemistry parameters and trout densities [27]. Variation in density estimates within sites was assessed for relationships with conductivity and water temperature for each species and for both juvenile and adult length classes. We compared conductivity to trout densities to estimate the effects of trace elements on the trout populations because conductivity was highly correlated with boron and arsenic (see Results) and conductivity data were available for each day of every population survey. Boron and arsenic concentrations were only determined once during each week of the population surveys and did not always correspond with the exact day that a site was surveyed. Significance was assigned at $p \leq 0.05$ for all tests.

Aquatic habitat at each site was surveyed during base flows (September and October 1997) using the habitat quality index (HQI), a procedure developed to evaluate habitat for fluvial trout streams in Wyoming [28]. Measurements of several habitat attributes were compared among sites, including stream discharge (late summer and annual variation), water velocity, trout cover, stream width, bank stability, substrate composition, water temperature, and nitrate-nitrogen concentrations [29]. The HQI ratings range from zero to four, with four being the best. Ratings of nitrate-nitrogen concentrations were based on mean values during the one-year study period [28]. Attribute measurements were rated according to the HQI method, and an estimate of the number of habitat units for each site was computed using Model II. Two estimates of habitat quality were calculated for each site because of the extreme variation in water temperature among sites. One estimate used actual water temperatures to rate the maximum summer water temperature attribute, whereas the second estimate set the rating for the maximum summer temperature at three among all sites. A rating of three for the maximum summer temperature was used because this is the most common rating for mountain streams in Wyoming.

RESULTS

Water chemistry

Conductivity was highest and most variable in Fairy Creek (398–620 μS/cm) and the hot site (230–492 μS/cm). The high-

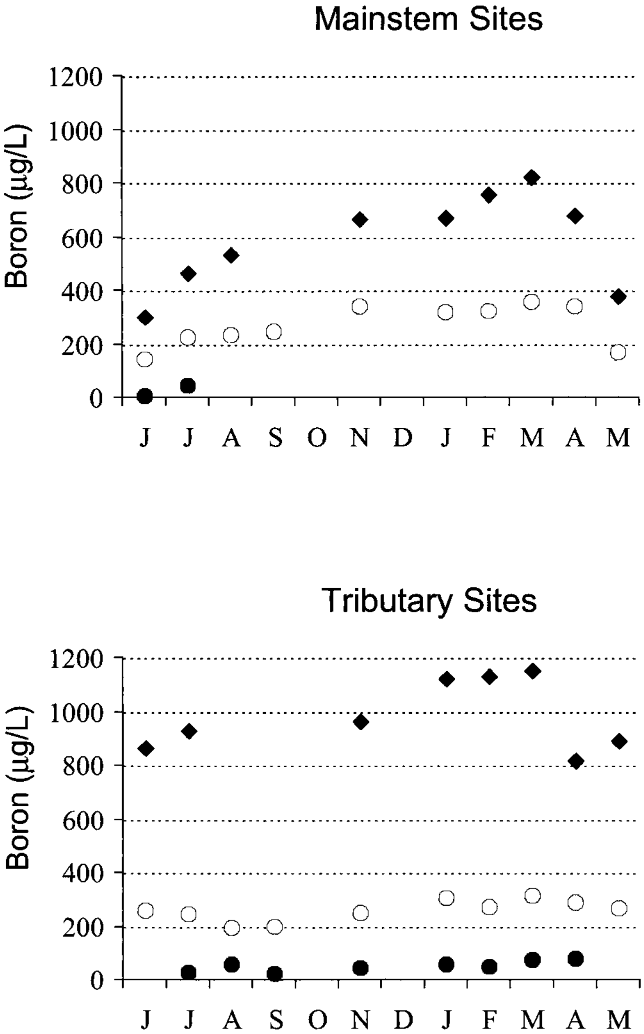

Fig. 2. Total boron concentrations measured at the study sites in the Firehole River (WY, USA) and tributaries, June 1997 to June 1998. Concentrations at mainstem sites are shown for the (●) cold, (○) warm, and (♦) hot sites. Concentrations at tributary sites are shown as (●) Little Firehole River, (○) Iron Springs Creek, and (♦) Fairy Creek.

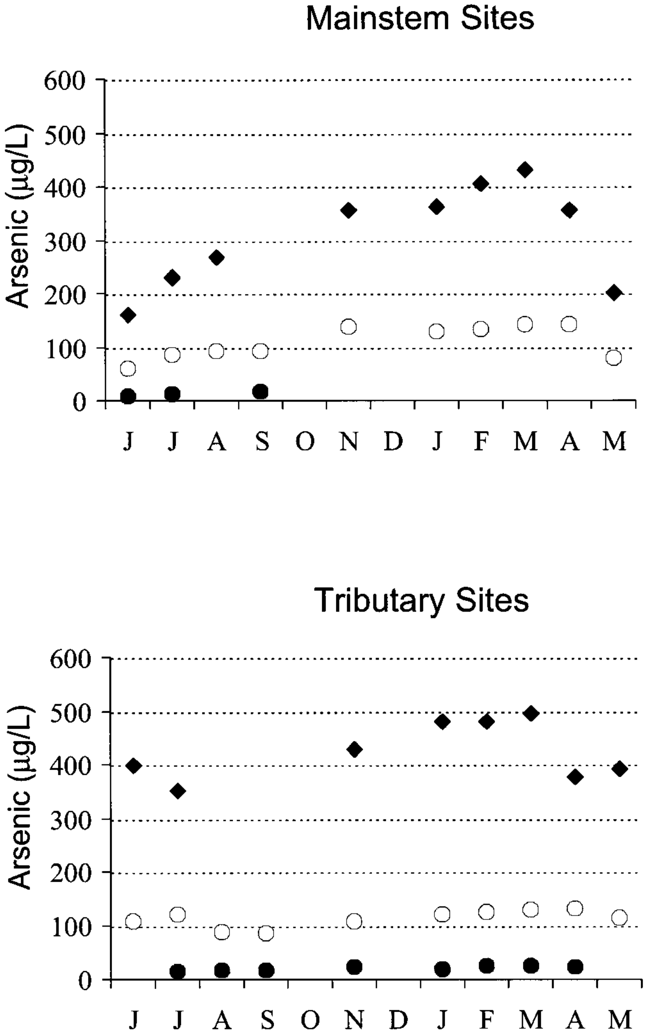

Fig. 3. Total arsenic concentrations measured at the study sites on the Firehole River (WY, USA) and tributaries, June 1997 to June 1998. Concentrations at mainstem sites are shown for the (●) cold, (○) warm, and (♦) hot sites. Concentrations at tributary sites are shown as (●) Little Firehole River, (○) Iron Springs Creek, and (♦) Fairy Creek.

est conductivity at these sites occurred during January 1998 in Fairy Creek and March 1998 in the hot site. Measurements of pH ranged from 6.3 to 9.0 among sites; the highest variability was observed at the Iron Springs Creek, Fairy Creek, and hot sites. Alkalinity ranged from 13 to 134 mg/L (as $CaCO_3$) among sites and was greatest and most variable in Fairy Creek (62–134 mg/L as $CaCO_3$) and the hot site (42–111 mg/L as $CaCO_3$). The TOC and DOC concentrations in the Fairy Creek and hot sites ranged from 18 to 25 mg/L during April 1998. However, TOC and DOC concentrations were <5 mg/L throughout the rest of the study at all sites.

Total ammonia concentrations were generally <100 µg N/L at all sites except Fairy Creek, where concentrations were mostly <100 µg N/L but ranged up to 256 µg/L. Un-ionized ammonia concentrations never exceeded 5 µg NH_3-N/L at any site except Fairy Creek. The unionized ammonia concentration in Fairy Creek was generally low (mean = 4.4 µg NH_3-N/L); however, one monthly water sample contained 40 µg NH_3-N/L.

Water in the Firehole River and tributaries was dominated by Na^+, HCO_3^-, and Cl^- (Table 1). Cation and anion concentrations were highest at the Fairy Creek and the hot site, often 10 to 20 times the concentrations observed at the other sites. The highest concentrations were observed during the winter from December 1997 to February 1998. Calcium, magnesium, and potassium concentrations were consistently low and did not contribute more than 10% to the total normality of cations. Nitrate, sulfate, and fluoride concentrations were also low and contributed less than 10% to the total normality of the anions.

Mean concentrations of total boron were 13 to 20 times greater in Fairy Creek and the hot site than in the cold and Little Firehole River sites (Fig. 2). The highest mean boron concentrations among sites were in the hot site (572 µg/L) and Fairy Creek (956 µg/L). The highest individual boron concentrations were observed during March 1998 at Fairy Creek (1,150 µg/L), the hot site (820 µg/L), the warm site (355 µg/L), and Iron Springs Creek (315 µg/L). Over 95% of the boron present was in the dissolved fraction.

Arsenic levels were elevated among the geothermally influenced sites, with the highest concentrations at the Fairy Creek and hot sites (Fig. 3). Total arsenic concentrations ranged from 160 to 500 µg/L at the Fairy Creek and hot sites,

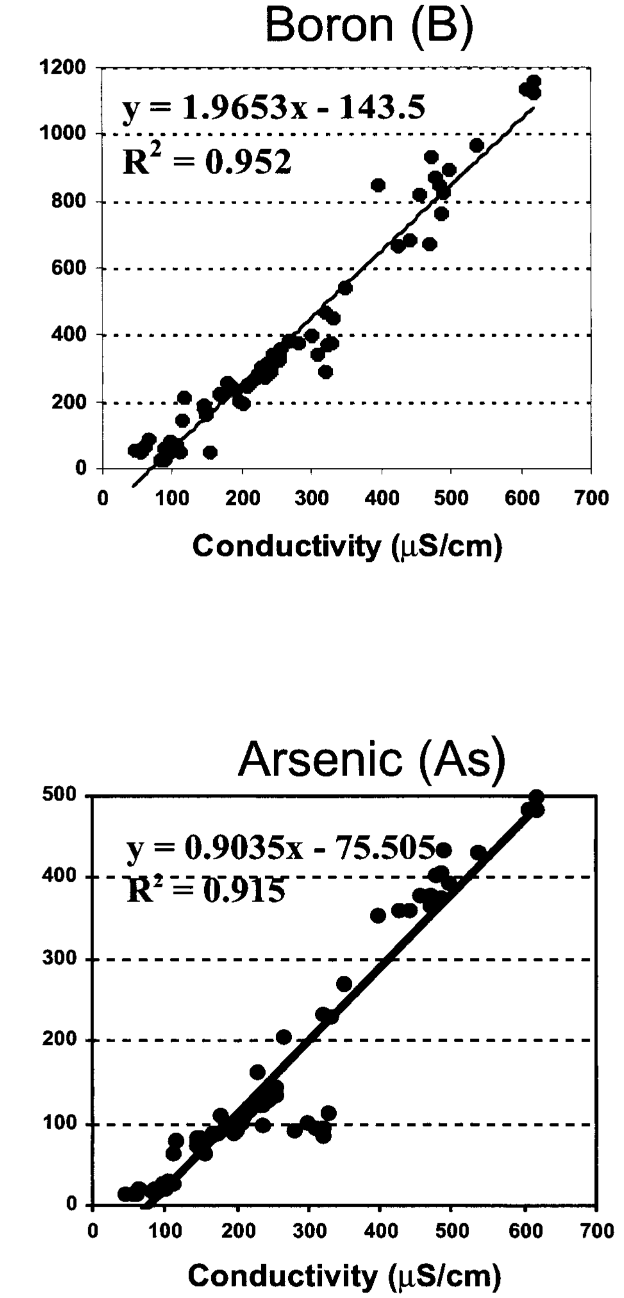

Fig. 4. Significant ($p < 0.05$) linear regressions of boron and arsenic concentrations with specific conductivity in the study sites on the Firehole River (WY, USA) and tributaries, June 1997 to June 1998. Values are in μg/L.

compared with 10 to 25 μg/L at the cold and Little Firehole River sites. Mean concentrations of total arsenic were also elevated at the Iron Springs Creek and warm sites (60–140 μg/L) but were lower than concentrations at the Fairy Creek and hot sites. Over 95% of the arsenic present was in the dissolved fraction. Additionally, boron and arsenic concentrations were significantly correlated with conductivity ($p < 0.01$, $R^2 > 0.90$; Fig. 4).

Arsenic speciation indicated that the majority of arsenic was usually present as As(V) at all sites [25]. Concentrations of As(III) were almost always measured below detection limits, but when As(III) concentrations were above detection limits, the species were present in close to equal proportions (55% as As(V), standard error of the mean [SEM] = 4%). Concentrations of As(V) and As(III) were highest at the Fairy Creek and hot sites, ranging from 50 to 250 μg As(III)/L and 110 to 380 μg As(V)/L. Concentrations of As(III) at the Iron Springs Creek and warm sites ranged from <35 to 100 μg/L, and concentrations of As(V) ranged from <35 to 140 μg/L. Concentrations of As(III) and As(V) at the Little Firehole River and cold sites were almost always below the detection limit of 35 μg/L.

Chromium concentrations were usually below detection limits at all sites; however, concentrations were sporadically elevated (up to 250 μg/L) in the Fairy Creek, warm, and hot sites [25]. Additionally, selenium concentrations were low and remained <3 μg/L at all sites.

Water temperatures were consistently higher in the downstream mainstem sites of the Firehole River than in upstream sites (Fig. 5). Temperatures at the warm and hot sites averaged 5 and 10°C higher, respectively, than the cold site. Temperature regimes were relatively similar between the following pairs of sites: the cold site and Little Firehole River; the warm site and Iron Springs Creek; and the hot site and Fairy Creek. The maximum water temperatures observed among these sites were, at Fairy Creek, 30°C (July 21, 1997); at the hot site, 26°C (July 21, 1997); at the warm site, 22°C (July 16, 1997); and at Iron Springs Creek, 25°C (July 15, 1997).

Data collected at the Sentinel Creek and Nez Perce Creek sites are not presented because underwater visibility precluded fish surveys in Sentinel Creek and low densities of salmonids were observed in Nez Perce Creek because of poor habitat.

Salmonids

Rainbow trout dominated the study sites with higher trace element concentrations and water temperatures, whereas brown trout dominated at the sites with lower trace element concentrations and lower temperatures (Table 2). Rainbow trout were present across a wide range of trace element concentrations, whereas brown trout densities decreased significantly as trace element concentrations increased among sites ($p < 0.01$; Fig. 6). Boron, arsenic, and other trace element concentrations were highest at Fairy Creek (Fig. 7) and the hot sites (Fig. 8), where rainbow trout were the dominant species and brown trout were rarely observed. Rainbow trout density among all study sites increased with stream temperature ($p < 0.01$); however, the trend among brown trout was the opposite, with higher brown trout densities being observed with lower temperatures (Fig. 9).

A similar trend was observed in the spatial distribution of species during spawning. Thirty-four redds were observed in the upper Firehole River (including the cold site) and in the Little Firehole River during November 1997. Seventy-five redds were observed in the lower Firehole River (including the hot site) and 45 redds were observed in Fairy Creek during December 1997 and January 1998. Fry were collected from these sites from March through May 1998 and were identified to determine which trout species had successfully spawned in each area. The 35 fry collected from the Little Firehole River were both brown trout (63%) and rainbow trout (37%). The fry collected from the hot site and Fairy Creek were dominated by rainbow trout, with 95% of the 74 fry collected at these sites being identified as rainbow trout.

Habitat quality was similar among sites with the exception of water temperature (Table 3). Habitat quality ranged from 250 to 388 habitat units (predicted standing stock of trout from 260–400 kg/ha) among sites when the rating for maximum

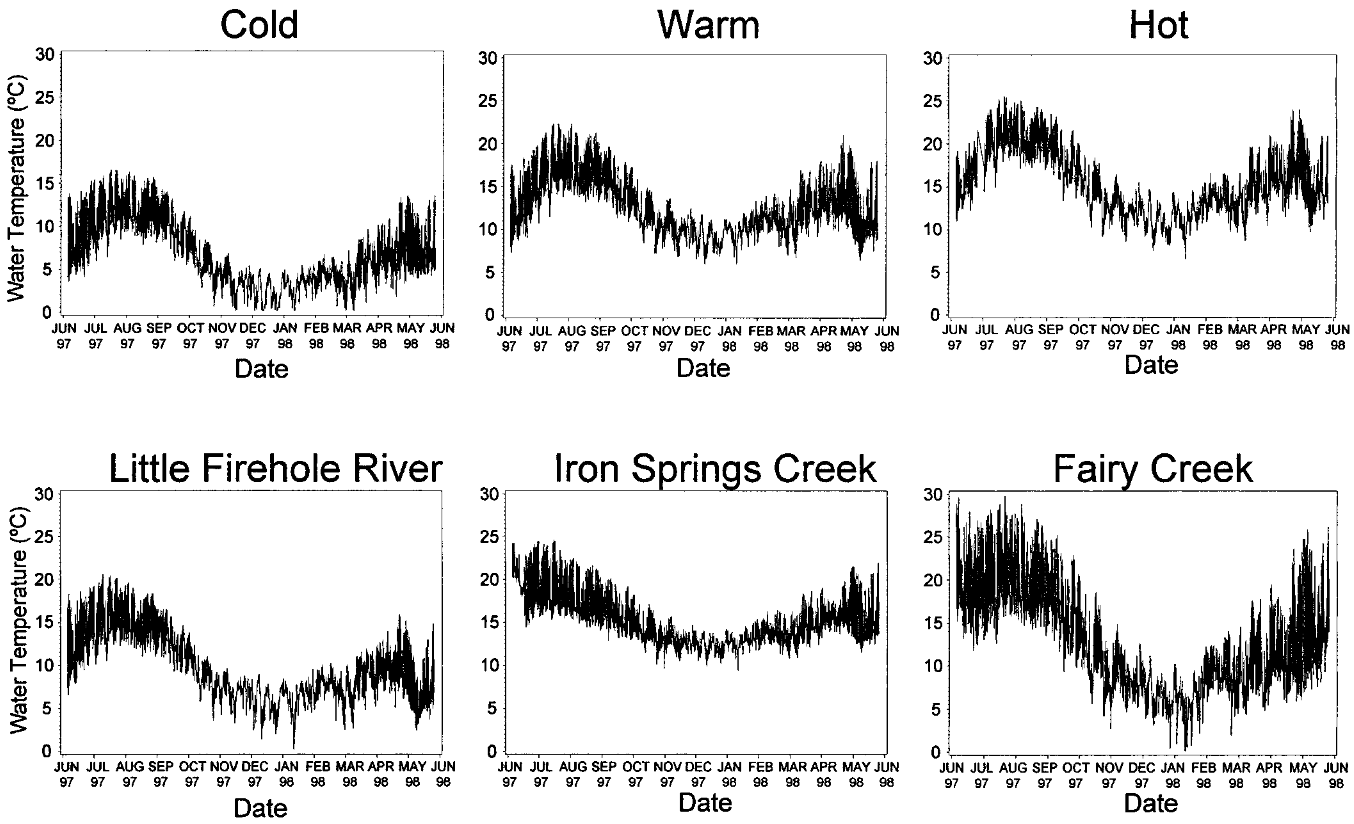

Fig. 5. Hourly water temperatures at study sites in the Firehole River (WY, USA) and tributaries, June 1997 to June 1998.

summer temperature was the same among sites (Table 4). When actual maximum summer temperatures were used in the model, the rating of habitat quality at the Iron Springs Creek, hot, Fairy Creek, and Nez Perce Creek sites decreased. Predicted standing stocks of trout were much less when actual temperatures were used because the model rates these high temperatures as being very poor for trout. Predicted standing stock decreased from 268 to 55 kg/ha at the hot site and from 259 to 2 kg/ha in Fairy Creek when actual temperatures were used. The ratings from the HQI model demonstrated that habitat quality was relatively similar among sites but elevated water temperatures in the geothermally influenced sites substantially reduce habitat quality for trout.

DISCUSSION

Our study demonstrates that rainbow trout in the Firehole River basin tolerate high concentrations of boron, arsenic, and other trace elements and elevated temperatures in geothermally influenced waters. Boron concentrations at the most geothermally influenced site ranged from 800 to 1,200 μg/L during our study, and these concentrations are consistent with the limited historical data for this basin [3]. In fact, rainbow trout densities tended to increase among sites as the influence of geothermal inputs increased. The combinations of elevated concentrations of boron, arsenic, other trace elements, and elevated temperatures in Fairy Creek and the hot site did not appear to negatively impact rainbow trout.

Table 2. Relative composition of rainbow trout and brown trout juveniles and adults in the Firehole River drainage (WY, USA) among eight observation periods combined from July to November 1997

Site	Adults		Juveniles	
	Rainbow trout (%)	Brown trout (%)	Rainbow trout (%)	Brown trout (%)
Cold	20.5	79.5	1.2	98.8
Warm	48.0	52.0	39.6	60.4
Hot	88.0	12.0	93.5	6.5
Little Firehole River	30.0	70.0	16.2	83.8
Iron Springs Creek	71.4	28.6	70.8	29.2
Fairy Creek	100.0	0.0	100.0	0.0

Brown trout in the Firehole River basin seemed to be limited by geothermal inputs. Brown trout were rare or absent in the lower Firehole River and Fairy Creek, where trace element concentrations and temperatures were the highest. Brown trout densities decreased among sites as trace element concentrations and temperature increased, and they were not found to spawn in the areas with the highest trace element concentrations and temperatures. Low densities of brown trout in geothermally influenced areas in the Firehole River have been reported previously [30,31].

Water temperatures exceeding the tolerance limit of embryos may have limited the distribution of brown trout in the Firehole River basin [25]. A review of the literature indicated differences in embryonic temperature tolerances between brown trout and rainbow trout. Brown trout embryos appear to survive at temperatures of 1 to 11°C [32–35], whereas rainbow trout embryos survive at 5 to 15°C [36–39]. Water temperatures typically exceed 11°C in Iron Springs Creek, Fairy Creek, the warm site, and the hot site during most of the year (Fig. 5) and might impact survival of brown trout embryos. Brown trout fry were rarely observed in collections from the spawning areas in Fairy Creek and the hot site.

Although temperature may be a factor determining the dis-

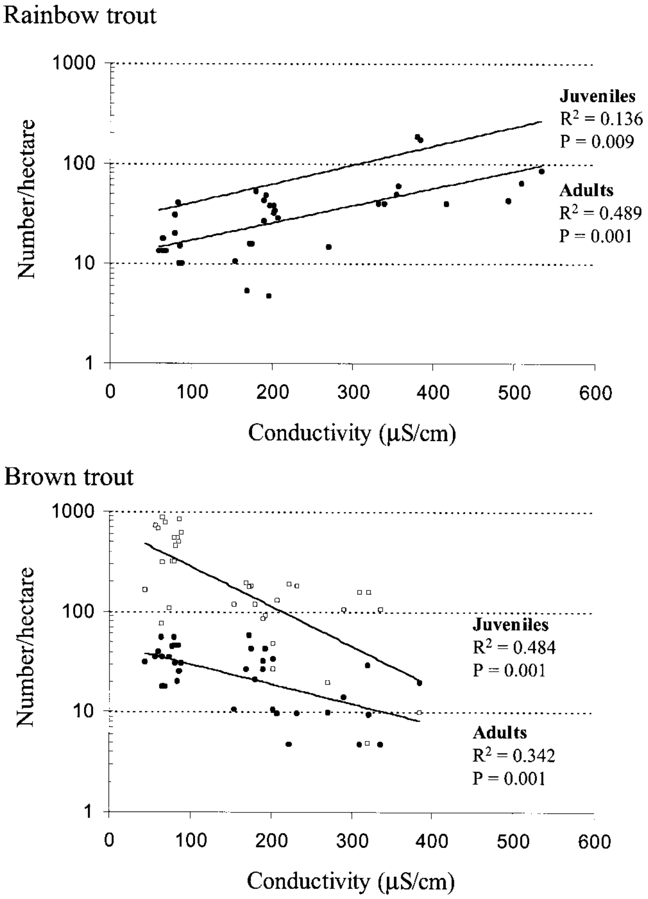

Fig. 6. Juvenile (○) and adult (●) trout densities (number per hectare; log scale) versus specific conductivity for population surveys conducted at study sites in the Firehole River and tributaries (WY, USA), 1997.

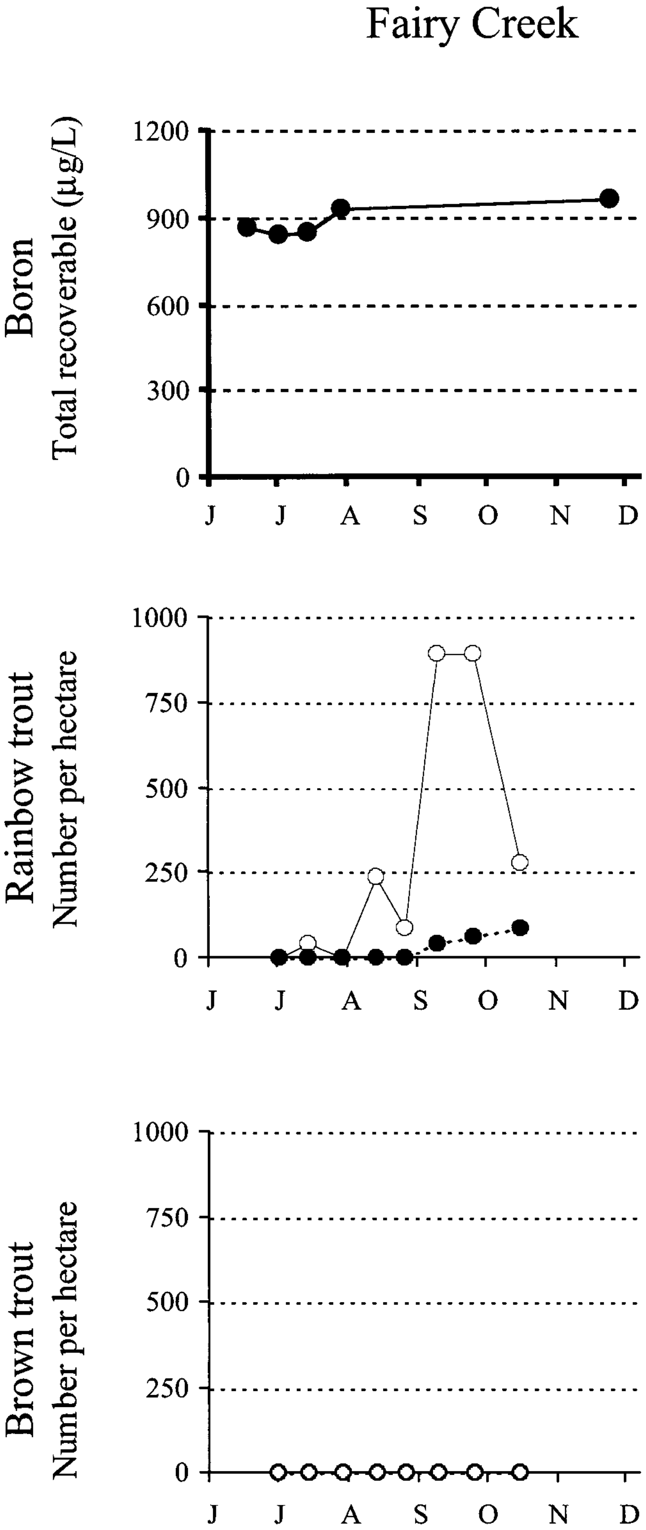

Fig. 7. Boron concentrations and densities of juvenile (○) and adult (●) trout at Fairy Creek in the Firehole River basin (WY, USA), 1997.

tribution of brown trout in the Firehole River basin, we cannot exclude the possibility that species-specific differences in trace element tolerances may also affect brown trout distributions. In our study, brown trout densities decreased when boron concentrations were above 400 µg/L and arsenic concentrations were above 200 µg/L. Tolerance of contaminants can vary greatly among salmonids [40–42]. Laboratory toxicity studies of boron on aquatic organisms have shown that freshwater fishes have a wide range of sensitivity to boron [43]. Kaya [30] reported reproductive abnormalities among a small group of brown trout collected in geothermally influenced waters in the Firehole River, including prespawning atresia in ova of females and immature gonads in 50% of the adult-sized males. Although these effects were attributed to high temperature, the potential role of trace element toxicity cannot be excluded.

Rainbow trout have shown the greatest sensitivity among aquatic organisms in aqueous-exposure tests [5,43,44]; however, brown trout have not been tested and the few studies of boron toxicity on rainbow trout embryos have not produced consistent results. The embryo–larval lifestage of rainbow trout was the most sensitive to acute lethal boron exposures [45]. The lowest observed effect concentrations (LOEC) for rainbow trout exposed to boron ranged from 100 to 18,000 µg/L, where concentrations of at least 1,000 µg/L were required to consistently cause effects on survival [5,43]. Birge and Black [46,47] and Birge et al. [48] observed boron-induced teratogenesis in rainbow trout embryos at 1 µg/L, but Rowe et al. [49] did not observe effects on embryos until boron concentrations were 10,000 µg/L. We are not aware of any boron toxicity data for brown trout or studies to assess sublethal effects of boron exposure on salmonids.

Sublethal concentrations of contaminants can cause behavioral avoidance [50,51], reduce fitness [52], and disrupt endocrine systems in salmonids [53]. Boron toxicity has been

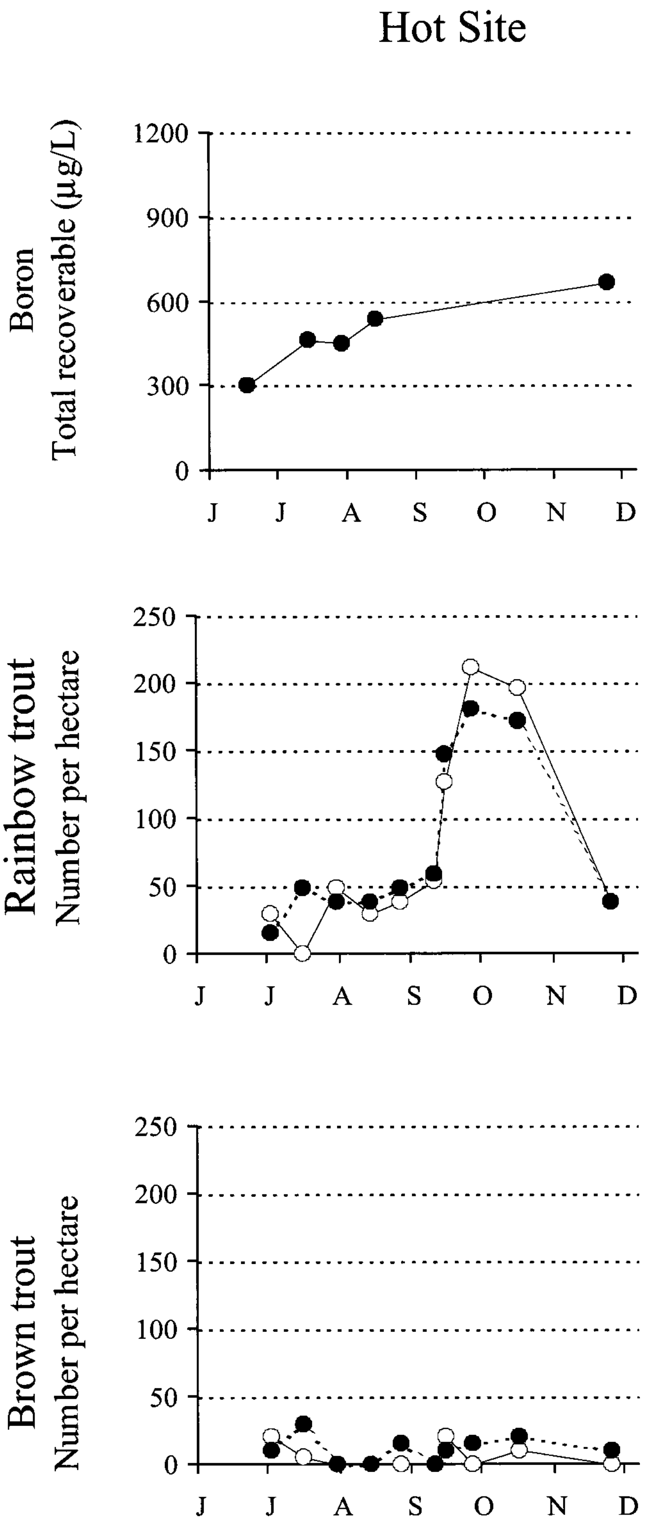

Fig. 8. Boron concentrations and densities of juvenile (○) and adult (●) trout at the hot site in the Firehole River basin (WY, USA), 1997.

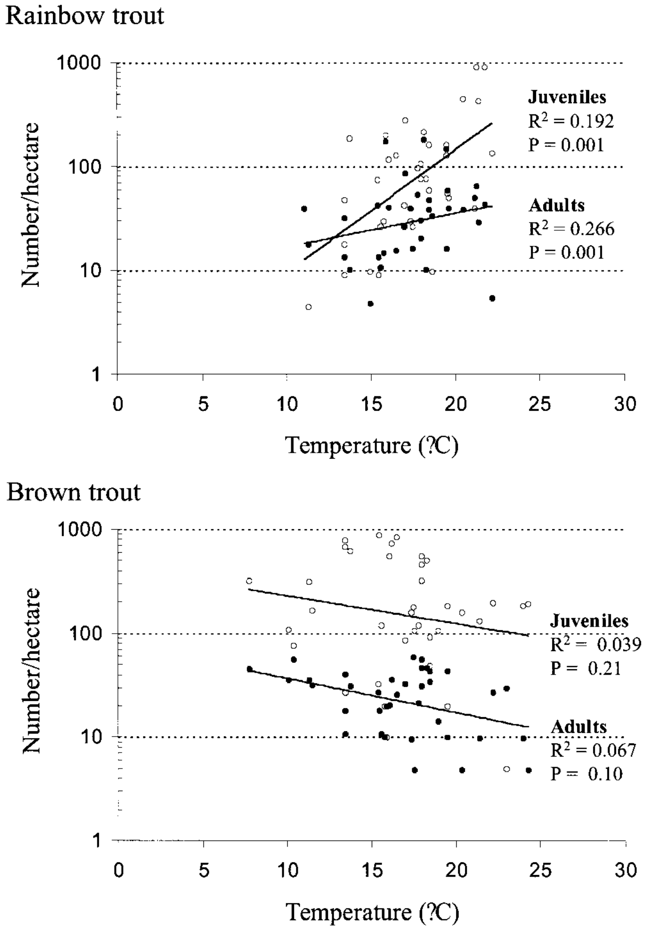

Fig. 9. Juvenile (○) and adult (●) trout densities (number per hectare; log scale) versus water temperature for population surveys conducted at study sites in the Firehole River and tributaries (WY, USA), 1997.

studied in other vertebrates and repeatedly shows testicular toxicity and other effects on reproductive systems [54–57]. Decreased fecundity in bluegill (*Lepomis macrochirus*) exposed to sublethal boron concentrations in the Salt Slough, California, have been observed, but other factors may have also influenced reproductive health in these fish [58]. Trout in the Firehole River may be additionally exposed to elevated trace element concentrations via the food web. Boron concentrations up to 3,500,000 μg/L have been observed in aquatic plants exposed to agricultural runoff [59], and elevated boron concentrations have been observed in whole-body tissue samples from bluegill and common carp (*Cyprinus carpio*) exposed to elevated boron in agricultural runoff [60]. Salmonids in the Firehole River feed mostly on immature benthic invertebrates, emerging insects, and mollusks [61,62]. Future toxicity studies should address the potential of this route for increased exposure and assess sublethal effects of boron exposure on fish health and reproductive physiology.

The highest arsenic concentrations observed in the Firehole River basin approached the U.S. EPA water quality criterion for surface waters. The U.S. EPA's chronic ambient water quality criterion for the protection of aquatic organisms for As(III) is 190 μg/L [63]. Aqueous exposure studies of rainbow trout embryos to As(III) have demonstrated an LC50 (lethal concentration to 50% of test subjects) of 540 μg/L [64]. Concentrations of As(III) exceeded the chronic ambient water quality criterion in Fairy Creek (208 μg/L) and in the hot site (256 μg/L) but were below acute ambient water quality criterion and LC50 values for rainbow trout. As(III) is more toxic than As(V) to aquatic organisms; however, As(V) is acutely toxic to rainbow trout at higher concentrations of 1,800 to 3,600 μg/L [65]. The highest concentrations of arsenic occurred during the late fall and winter, concurrent with low flows. The highest As(V) concentrations (377 μg/L) were below the toxic thresholds. Arsenic has been observed to cause depression of

Table 3. Habitat quality data used in the habitat quality index (HQI) evaluations of study sites in the Firehole River and tributaries, Yellowstone National Park (WY, USA), 1997[a]

	Site							
HQI parameters	Cold	LFHR	SC	Warm	Iron	NP	FC	Hot
Late summer stream flow (%)	96	97	91	113	116	95	96	101
Rating	4	4	4	4	4	4	4	4
ASFV (annual stream flow variation)	2.9	3.6	1.3	2.3	1.6	1.7	1.4	1.7
Rating	4	4	4	4	4	4	4	4
Maximum summer stream temperature (°C)	16.5	20.6	21.4	22.3	24.6	25.6	29.8	25.6
Rating	4	3	3	2	1	1	0	1
Mean nitrate-nitrogen (mg/L)	0.2	0.3	0.8	0.3	0.3	0.2	0.2	0.2
Rating	4	3	2	3	3	4	4	4
Cover (% surface area)	13	14	33	47	6	4	11	6
Rating	1	1	2	3	0	0	1	0
Eroding stream banks (% surface area)	0	1	4	0	0	0	7	0
Rating	4	4	4	4	4	4	4	4
Substrate (% surface area)	0	2	20	18	31	10	13	35
Rating	2	2	3	4	4	3	2	4
Water velocity (cm/s)	55	58	59	130	62	49	33	137
Rating	4	4	4	3	4	4	3	3
Stream width (m)	13.5	9.0	2.9	18.3	10.7	11.9	2.7	21.9
Rating	3	3	2	2	3	3	2	2

[a] LFHR = Little Firehole River; Iron = Iron Springs Creek; FC = Fairy Creek; SC = Sentinel Creek; and NP = Nez Perce Creek; cold, warm, hot = mainstem sites.

Table 4. Predictions of standing stock and habitat quality units from the habitat quality index (HQI) evaluations of study sites in the Firehole River and tributaries, Yellowstone National Park (WY, USA), 1997[a]

	Site					
HQI estimates	Cold	Warm	Hot	LFHR	Iron	FC
Based on actual temperatures						
Standing stock of salmonids (kg/ha)	524	219	57	277	57	2
Habitat quality units	505	211	55	267	55	2
Based on constant acceptable temperatures						
Standing stock of salmonids (kg/ha)	332	403	268	277	268	259
Habitat quality units	320	388	258	267	258	250

[a] Cold, warm, hot = mainstem sites; LFHR = Little Firehole River; Iron = Iron Springs Creek; and FC = Fairy Creek.

growth and impaired feeding in rainbow trout fed arsenic-contaminated diets [66]. Again, we are not aware of arsenic toxicity data for brown trout to make comparisons among species-specific tolerances. However, there is potential for arsenic to cause toxic effects on aquatic organisms in the Firehole River and should be investigated in more detail.

Our study demonstrates that rainbow trout in the Firehole River tolerated elevated boron concentrations. Brown trout may be in low abundance at geothermally influenced sites because of differences in temperature tolerances; however, they may also be more sensitive to boron, arsenic, and other trace elements than rainbow trout. Research is needed to assess if there are species-specific differences in tolerance to elevated boron, arsenic, and other trace elements among salmonids because they tend to be the most sensitive aquatic organisms. Furthermore, trophic transfer and sublethal effects of boron on fish health should be investigated. These studies will help refine surface water quality criteria for trace elements and protect aquatic organisms and their ecosystems.

Acknowledgement—We thank Wilbur Mauck, David Harper, and staff, U.S. Geological Survey, and Connie Boese, Steve Boese, Marjorie Brooks Lovvorn, James Drever, and staff, University of Wyoming. We are grateful to John Varley, Daniel Mahony, and Aquatic Resources personnel of the U.S. National Park Service, Yellowstone National Park, and to Les Inafuku, Old Faithful Rangers, and the Old Faithful Maintenance crew. Funding was provided by Procter & Gamble, D. Scott Dyer; Borax Europe, Jonathan B. Rainer; Unilever United Kingdom, Central Resources, A.A. McKinnon; and CEFIC European Chemical Industry Council, L. Le Dore.

REFERENCES

1. Zeikus JG, Brock TD. 1972. Effects of thermal additions from the Yellowstone geyser basins on the bacteriology of the Firehole River. *Ecology* 53:283–290.
2. Kaeding LR. 1996. Summer use of coolwater tributaries of a geothermally heated stream by rainbow and brown trout, *Oncorhynchus mykiss* and *Salmo trutta*. *Am Midl Nat* 135:238–292.
3. Meyer JS, Boelter AM, Woodward DF, Goldstein JN, Farag AM, Hubert WA. 1998. Relationships between boron concentrations and trout in the Firehole River, Wyoming. *Biol Trace Elem Res* 66:167–184.
4. Committee of Consultants on San Joaquin River Water Quality Objectives. 1988. The evaluation of water quality criteria for selenium, boron, and molybdenum in the San Joaquin River basin. *Series on Drainage, Salinity, and Toxic Constituents,* No. 4. University of California, Davis, CA.
5. Black JA, Barnum JB, Birge WJ. 1993. An integrated assessment of the biological effects of boron to the rainbow trout. *Chemosphere* 26:1383–1413.
6. Roberts M. 1995. EU cites health effects in proposed boron cut. *Chem Week* 156:15.
7. Butterwick L, de Oude N, Raymond K. 1989. Safety assessment of boron in aquatic and terrestrial environments. *Ecotoxicol Environ Saf* 17:339–371.
8. Hamilton SJ, Buhl KJ. 1990. Acute toxicity of boron, molyb-

denum and selenium to fry of chinook salmon and coho salmon. *Arch Environ Contam Toxicol* 19:366–373.
9. Hamilton SJ, Wiedmeyer RH. 1990. Concentrations of boron, molybdenum, and selenium in chinook salmon. *Trans Am Fish Soc* 119:500–510.
10. Druse SA, Glass WR, Ritz GF, Smalley ML. 1993. Water resources data for Wyoming, water year 1993. Water Report WY-93-1. U.S. Geological Survey, Cheyenne, WY.
11. U.S. Geological Survey. Water records 1983–1993. Water Resources Division, Helena, MT.
12. Allen ET, Day AL. 1935. Hot springs of the Yellowstone National Park. Publication 466. Carnegie Institute, Washington, DC.
13. Boylen CW, Brock TD. 1973. Effects of thermal additions from the Yellowstone geyser basins on the benthic algae of the Firehole River. *Ecology* 54:1282–1291.
14. Thompson JM. 1979. Arsenic and fluoride in the Upper Madison River system: Firehole and Gibbon Rivers and their tributaries, Yellowstone National Park, Wyoming, and southeast Montana. *Environ Geol* 3:13–21.
15. Argyle RL. 1966. Thermal dispersion in lotic waters of Yellowstone National Park. MS thesis. Colorado State University, Ft. Collins, CO, USA.
16. Wright JC, Horrall RM. 1967. Heat budget studies on the Madison River, Yellowstone National Park. *Limnol Oceanogr* 12:578–583.
17. Burkhalter DE. 1979. Thermal and hydraulic characteristics of a geothermally influenced trout stream: The Firehole River of Yellowstone National Park. PhD thesis. Montana State University, Bozeman, MT, USA.
18. Benson NG, Cope OB, Bulkley RV. 1959. Fishery management studies on the Madison River System in Yellowstone National Park. Special Scientific Report—Fisheries 307. U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC.
19. Fromm RJ. 1941. An open history of fish and fish planting in Yellowstone National Park. U.S. Department of the Interior, National Park Service, Yellowstone National Park, WY.
20. Varley JD. 1981. A history of fish stocking activities in Yellowstone National Park between 1881 and 1980. No. 35. Yellowstone National Park, WY, USA.
21. Jones RD, Andrascik R, Carty DG, Kaeding LR, Kelly BM, Mahony DL, Olliff T. 1992. Annual project technical report for 1991: Fishery and Aquatic Management Program, Yellowstone National Park. U.S. Department of the Interior, Fish and Wildlife Service, Fisheries and Federal Aid, Yellowstone National Park, WY.
22. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1985. *Standard Methods for the Examination of Water and Wastewater,* 16th ed. American Public Health Association, Washington, DC.
23. Ivancic I, DeGobbis D. 1984. An optimal manual procedure for ammonia analyses in natural waters by the indophenol blue method. *Water Res* 18:1143–1147.
24. Emerson K, Russo RC, Thurston RV. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J Fish Res Board Can* 32:2379–2383.
25. Goldstein JN. 1999. Effects of geothermal effluents on rainbow trout and brown trout in the Firehole River, Yellowstone National Park, Wyoming. MS thesis. University of Wyoming, Department of Zoology and Physiology, Laramie, WY, USA.
26. Stauffer RE. 1980. Molybdenum blue applied to arsenic and phosphorus determinations in fluoride- and silica-rich geothermal waters. *Environ Sci Technol* 14:1475–1481.
27. SAS Institute. 1996. *SAS User's Guide: Statistics,* Ver 6. Cary, NC, USA.
28. Binns NA, Eiserman FM. 1979. Quantification of fluvial trout habitat in Wyoming. *Trans Am Fish Soc* 108:215–228.
29. McMahon TE, Zale AV, Orth DJ. 1996. Aquatic habitat measurements. In Murphy BR, Willis DW, eds, *Fisheries Techniques,* 2nd ed. American Fisheries Society, Bethesda, MD, pp 83–120.
30. Kaya CM. 1977. Reproductive biology of rainbow and brown trout in a geothermally heated stream: The Firehole River of Yellowstone National Park. *Trans Am Fish Soc* 106:354–361.
31. Koch ED. 1990. Effects of temperature and angling on brown and rainbow trout populations in the Firehole River, Yellowstone National Park. MS thesis. Idaho State University, Pocatello, ID, USA.
32. Embody GC. 1934. Relation of temperature to the incubation period of eggs of four species of trout. *Trans Am Fish Soc* 64:281–292.
33. Humpesch UH. 1985. Inter- and intra-specific variation in hatching success and embryonic development of five species of salmonids and *Thymallus thymallus. Arch Hydrobiol* 104:129–144.
34. Jungwirth M, Winkler H. 1984. The temperature dependence of embryonic development of grayling (*Thymallus thymallus*), danube salmon (*Hucho hucho*), Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta fario*). *Aquaculture* 38:315–327.
35. Stonecypher RW, Hubert WA, Gern WA. 1994. Effect of reduced incubation temperatures on survival of trout embryos. *Prog Fish Cult* 56:180–184.
36. Danzmann RG, Ferguson MM. 1988. Temperature-dependent genotypic selection and embryonic survival of rainbow trout. *Biochem Genet* 26:69–81.
37. Hokanson KEF, Kleiner CF, Thorslund TW. 1977. Effects of constant temperature and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, *Salmo gairdneri. J Fish Res Board Can* 34:339–348.
38. Kwain W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature. *J Fish Res Board Can* 32:397–402.
39. Timoshina LA. 1972. Embryonic development of the rainbow trout (*Salmo gairdneri irideus* Gibb.) at different temperatures. *J Ichthyol* 12:425–432.
40. Atchison GJ, Henry MG, Sandheinrich MB. 1987. Effects of metals on fish behavior: A review. *Environ Biol Fish* 18:11–25.
41. Marr J, Lipton J, Cacela D. 1995. Fisheries toxicity injury studies, Blackbird Mine site, Idaho (contract 50-DGNC-1-00007, task order 50084). National Oceanic and Atmospheric Administration, Washington, DC, USA.
42. Hansen JA, Woodward DF, Little EE, DeLonay AJ, Bergman HL. 1999. Behavioral avoidance: Possible mechanisms for explaining the abundance and distribution of trout species in a metal-impacted river. *Environ Toxicol Chem* 18:313–317.
43. European Centre for Ecotoxicology and Toxicology of Chemicals. 1997. Ecotoxicology of some inorganic borates. Special Report 11. Brussels, Belgium.
44. Eisler R. 1990. Boron hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85 (1.20). U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD.
45. Eckhert C. 1997. Embryonic trout growth and boron exposure. *FASEB* 11:A406.
46. Birge WJ, Black JA. 1977. Sensitivity of vertebrate embryos to boron compounds. EPA 560/1-76-008. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, DC.
47. Birge WJ, Black JA. 1981. Toxicity of boron to embryonic and larval stages of largemouth bass (*Micropterus salmoides*) and rainbow trout (*Salmo gairdneri*). Completion Report. Procter & Gamble, Cincinnati, OH, USA.
48. Birge WJ, Black JA, Westermann AG, Short TM, Taylor SB, Parekh MC. 1984. Toxicity of boron to embryonic and larval stages of rainbow trout (*Salmo gairdneri*) exposed in reconstituted and natural waters. Completion Report. Procter & Gamble, Cincinnati, OH, USA.
49. Rowe RI, Bouzan C, Nabili S, Eckhert CD. 1998. The response of trout and zebrafish to low and high boron concentrations is U-shaped. *Biol Trace Elem Res* 66:261–270.
50. Woodward DF, Goldstein JN, Farag AM, Brumbaugh WG. 1997. Cutthroat trout avoidance of metals and conditions characteristic of a mining waste site: Coeur d'Alene River, Idaho. *Trans Am Fish Soc* 126:699–706.
51. Goldstein JN, Woodward DF, Farag AM. 1999. Movements of adult chinook salmon during spawning migration in a metals-contaminated system, Coeur d'Alene River, Idaho. *Trans Am Fish Soc* 128:121–129.
52. Farag AM, Woodward DF, Brumbaugh W. 1999. Dietary effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on cutthroat trout. *Trans Am Fish Soc* 128:578–592.
53. Heath AG. 1995. *Water Pollution and Fish Physiology,* 2nd ed. CRC, Boca Raton, FL, USA.
54. Ku WW, Chapin RE, Wine RN, Gladen BC. 1993. Testicular toxicity of boric acid (BA); relationship of dose to lesion development and recovery in the F344 rat. *Reprod Toxicol* 7:305–319.
55. Ku WW, Shih LM, Chapin RE. 1993. The effects of boric acid (BA) on testicular cells in culture. *Reprod Toxicol* 7:321–331.
56. Ku WW, Chapin RE. 1994. On the mechanism of testicular tox-

icity of boric acid in rats: In vivo and in vitro studies. *Environ Health Perspect* 102:99–105.
57. European Centre for Ecotoxicology and Toxicology of Chemicals. 1995. Reproductive and general toxicology of some inorganic borates and risk assessment for human beings. Technical Report 63. Brussels, Belgium.
58. Nakamoto RJ, Hassler TJ. 1992. Selenium and other trace elements in bluegills from agricultural return flows in the San Joaquin Valley, California. *Arch Environ Contam Toxicol* 22:88–98.
59. Schuler CA. 1987. Impacts of agricultural drainwater and contaminants on wetlands at Kesterson Reservoir, California. MS thesis. Oregon State University, Corvallis, OR, USA.
60. Saiki MK, May TW. 1988. Trace element residues in bluegills and common carp from the lower San Joaquin River, California, and its tributaries. *Sci Total Environ* 74:199–217.
61. Armitage KB. 1958. Ecology of the riffle insects of the Firehole River, Wyoming. *Ecology* 39:571–580.
62. Armitage KB. 1961. Distribution of riffle insects of the Firehole River, Wyoming. *Hydrobiologia* 17:152–174.
63. U.S. Environmental Protection Agency. 1987. Quality criteria for water: 1986. EPA 440/5-86-001. Washington, DC.
64. U.S. Environmental Protection Agency. 1980. Ambient water quality criteria for arsenic. EPA 440/5-80-021. Washington, DC.
65. McGeachy SM, Dixon DG. 1992. Whole-body arsenic concentrations in rainbow trout during acute exposure to arsenate. *Ecotoxicol Environ Saf* 24:301–308.
66. Cockell KA, Hilton JW. 1985. Chronic toxicity of dietary inorganic and organic arsenicals to rainbow trout (*Salmo gairdneri* R.). Federation proceedings. *FASEB* 44:938.