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[Article]

Comparison of Growth and Stress in Resident Redband Trout Held in Laboratory Simulations of Montane and Desert Summer Temperature Cycles

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Abstract.—Within their native range in western North America, resident redband trout *Oncorhynchus mykiss gairdneri* occupy stream habitat from high mountains to low desert. To better understand the temperature tolerance, growth, and stress physiology of native redband trout populations and compare the resilience and responses to reciprocal environments of stocks adapted to desert or montane conditions, we conducted controlled laboratory trials. We tested groups of age-0 progeny from naturally reproducing desert and montane fish stocks in temperature cycles that simulated the summer conditions typical in regional desert and montane stream habitats. The diel cycles ranged from 8°C to 16°C for montane treatments and from 18°C to 26°C for desert treatments, and our tests were repeated over 2 years. We evaluated survival, growth, feed efficiency, plasma cortisol, heat shock protein levels, and body proximate composition in samples of fish collected during and at the completion of the trials. All of the stocks tested had high survival under all conditions, regardless of their geographic origin. We found no differences consistently attributable to desert or montane origin. Growth rates and protein and lipid efficiencies varied among stocks, between temperature treatments, and between replicate years. We found that the expression of heat shock protein 70 (hsp70) was consistently higher in all stocks maintained at desert temperatures regardless of source, but the absolute quantity of proteins measured varied among populations. We conducted an additional short-term trial to evaluate the responses of different stocks to upper lethal temperature cycles that approached a daily maximum of 30°C. Although desert- and montane-adapted populations of redband trout were equally dynamic and adaptive in desert or montane diel temperature cycles, we conclude that the desert stocks will be more at risk from increasing temperatures and reduced stream flows in the summer months as climate changes.

Rainbow trout *Oncorhynchus mykiss* are native to western North America and their appropriate phylogeny and taxonomy is unresolved (e.g., Behnke 1992; Currens et al. 2007; Thurow et al. 2007). Native rainbow trout occurring east of the Cascade Range within the Columbia River basin are classified as redband trout *O. mykiss gairdneri* (Behnke 1992). In southern Idaho, Columbia River basin resident redband trout are native to the Snake River sub-basin below Shoshone Falls, and occupy habitat from high elevation, cool mountain streams to low elevation, desert streams (Schill et al. 2007).

During months of low flow in desert environments, afternoon water temperatures can reach 32°C for short periods (Idaho Department of Fish and Game, unpublished data). Various studies report the upper critical temperatures for strains of rainbow trout as 26.9–29.8°C, depending on acclimation temperature (Lee and Rinne 1980; Currie et al. 1998; Beitinger et al. 2000). However, Behnke (1992) and Zoellick (1999) both found actively feeding desert redband trout in water temperatures of 26–28°C and both investigators have suggested that desert redband trout may have evolved unique physiological mechanisms that enable them to withstand high temperatures. Despite these findings, few studies have addressed the growth, temperature tolerance, and dynamics of desert redband trout populations.

Many studies of temperature tolerance of salmonids focus on critical thermal maxima (CTM), incipient lethal temperature (ILT), or chronic lethal maxima (CLM) (Currie et al. 1998; Beitinger et al. 2000; Bear et al. 2007). While tests such as these are important to understand the generalized thermal tolerance of fish, they do not provide information regarding the response of salmonids to fluctuating temperatures, especially those observed in some desert environments that change up to 15°C during summer conditions. Although daily thermal cycles are part of the natural environment, few studies have been conducted to determine the effects of diel temperature fluctuations on the physiology of salmonids.

Several groups have petitioned the U.S. Fish and

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Wildlife Service (USFWS) to consider redband trout in the Snake River drainage upstream from Brownlee Dam and below Shoshone Falls as a distinct vertebrate population segment under the Endangered Species Act (U.S. Office of the Federal Register 1995) because of concern for the threats to geographically isolated and potentially unique populations of redband trout. However, petitions have been denied because insufficient information was provided to demonstrate that the interior redband trout of the middle Snake River desert comprised a distinct population segment. Presently, the USFWS considers the interior redband trout as a "species of concern" and seeks additional information regarding the status of, and threats to, the subspecies (U.S. Office of the Federal Register 1995; Rhew 2007).

Recent studies provide a more optimistic assessment of the resilience of desert redband stocks. Zoellick et al. (2005) determined that the mean density of selected desert populations of redband trout remained similar in southern Idaho desert streams between the 1970s and 1990s. Redband trout residing in remote desert streams have little fishing pressure and mortality rates are well below those for stocks in more accessible montane environments (Schill et al. 2007).

In this laboratory study we tested and evaluated the stress physiology and resiliency of age-0 wild stocks of desert and montane-adapted redband trout in simulated desert and montane diel water temperatures. In addition to determining growth and survival, we evaluated feed conversion efficiency, proximate composition of muscle tissues, production of heat-shock proteins, and plasma cortisol concentrations.

Methods

Source Streams, Gamete Collection, and Controls

We identified suitable accessible locations in southwest Idaho desert and montane streams (Figure 1) with endemic redband trout stocks presumed to have no hatchery influence based on the stocking records (Idaho Fish and Game, unpublished data available at fishandgame.idaho.gov/apps/stocking/). During the spring of 2006 and 2007, we sampled suitable streams from March through May to locate sexually mature fish from which to collect gametes.

Using a backpack electrofisher, we stunned and netted redband trout into a bucket containing a solution of approximately 50 mg/L tricaine methanesulfonate (Argent Chemicals, Redmond, Washington) for sedation. Eggs or milt from mature fish were collected and placed into individually labeled plastic bags, charged with oxygen, and transported to the University of Idaho fisheries wet laboratory on ice. We deployed multiple Hobo temperature loggers (Onset Computers, Bourne, Massachusetts) in shaded pools in all the source streams to record hourly water temperatures during the spring and summer, and compare thermal profiles with treatments applied to fish in the laboratory.

In 2006 and 2007, we collected gametes from redband trout from Shoofly Creek and Jump Creek, second-order desert tributaries of the Snake River originating in the Owyhee Mountains. In 2007, we collected gametes from the confluence of Cabin and Corral creeks, tributaries of the North Fork of the Owyhee River. In both years we collected gametes from fish in Keithly Creek, a second-order montane tributary to the Weiser River originating in the Hitt Mountains on the Payette National Forest. In 2006, high spring flows impeded collection of fish from other montane streams. In 2007, we collected gametes from fish in Big Pine Creek, a third-order montane tributary of the South Fork of the Payette River. To serve as a control comparison, we obtained fertilized diploid eggs from rainbow trout broodstock from Hayspur State Hatchery (Idaho Department of Fish and Game, Gannett), a hatchery stock founded from three source populations: coastal McCloud River (California) stocks introduced to Silver Creek, Idaho; Mount Lassen Hatchery strain (California), and indigenous redband trout from the Big Wood River, Idaho (Williams et al. 1996).

Fertilization of Gametes and Rearing of Fish

In the laboratory we combined gametes by source stream with single parent crosses of males with females and a matrix-mating process to maximize genetic variation (7–16 fish of each sex). The fertilized eggs were placed into a Heath incubator tray, water hardened in 100 mg/L iodophor for 1 h, and transferred to incubators held at water temperatures of 5–10°C.

After hatching, we transferred the alevins from individual source streams to separate troughs and fed them a trout starter diet (Rangen, Buel, Idaho). We moved swimming fry to circular tanks for additional rearing and fed fish Biodiet grower (BioOregon, Warrenton, Oregon) ad libitum, and manipulated the water temperatures of each stock to obtain uniform degree-days and similar fish sizes before testing.

Diel Montane and Desert Study Design

We compared the responses of each desert- or montane-adapted stock in replicated tanks held in simulated desert or montane diel temperature treatments. Trials lasted for 35 d to simulate a typical peak summer condition, and diel temperature cycles simulated conditions previously measured in the source montane and desert systems. Daily cycles were 9–16°C and 18–26°C for montane and desert simulations, respectively. We mixed different quantities of chilled,

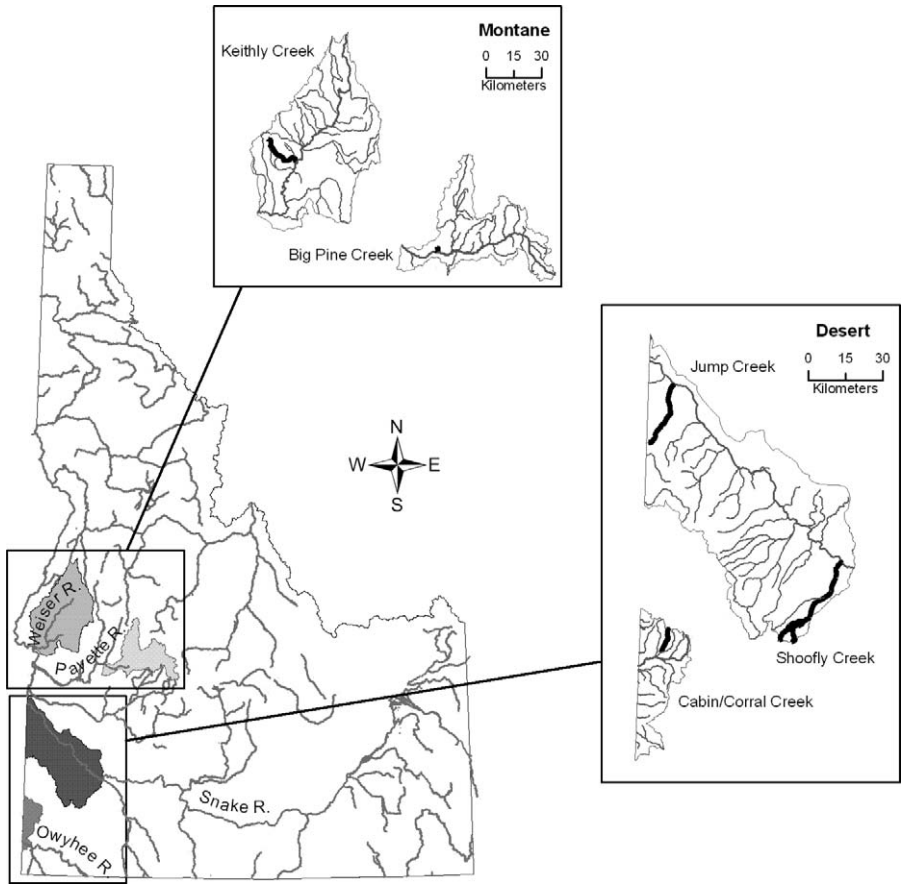

FIGURE 1.—Locations of the desert and montane streams from which gametes were collected from wild redband trout.

heated, and ambient water sources to create the thermal conditions in a common water supply for all montane or all desert simulations. Daily water temperatures were increased from the baseline low until mid-day; after 2 h at peak temperatures, water temperature was lowered gradually back to baseline by evening, and temperature profiles were recorded at 15-min intervals with Hobo data loggers. For acclimation during the first week of the trial, the peak temperature was kept 1–2°C below the target (Figure 2). To evaluate the magnitude of the daily temperature exposure, we computed the daily temperature units on an hourly basis for the source streams and for the laboratory treatments by selecting the temperatures at the start of every hour (Figure 2).

Specific pathogen-free dechlorinated water was supplied to each test tank as a flow-through system at 4–5 L/min. Supplemental compressed air was provided to each tank with an air stone to keep oxygen near saturation. The water level in 125-L circular test tanks was adjusted each year to hold fish in comparable densities within each test year (2006, 1.25 fish/L; 2007, 0.83 fish/L). To establish the starting fish weight in each tank, we weighed all fish (nearest gram) as a group. During each trial, we fed fish rations of BioDiet Grower twice daily to satiation. Satiation was determined by offering known quantities of feed to fish for 15 consecutive minutes, estimating uneaten food pellets in each tank, and calculating a net feed consumption for each tank of fish. We adjusted the photoperiod cycle for the trials daily to equal daylight for latitudes in southern Idaho.

Fish tanks were assigned at random to one of the two temperature treatments. In the first year, we tested fish from two desert stocks, one montane stock, and one hatchery stock. In the second year of tests, we placed fish from two desert stocks and two montane stocks into montane and desert treatments, and because of a limited number of test fish, we held fish from Cabin–Corral creeks only in desert cycles. We did not test

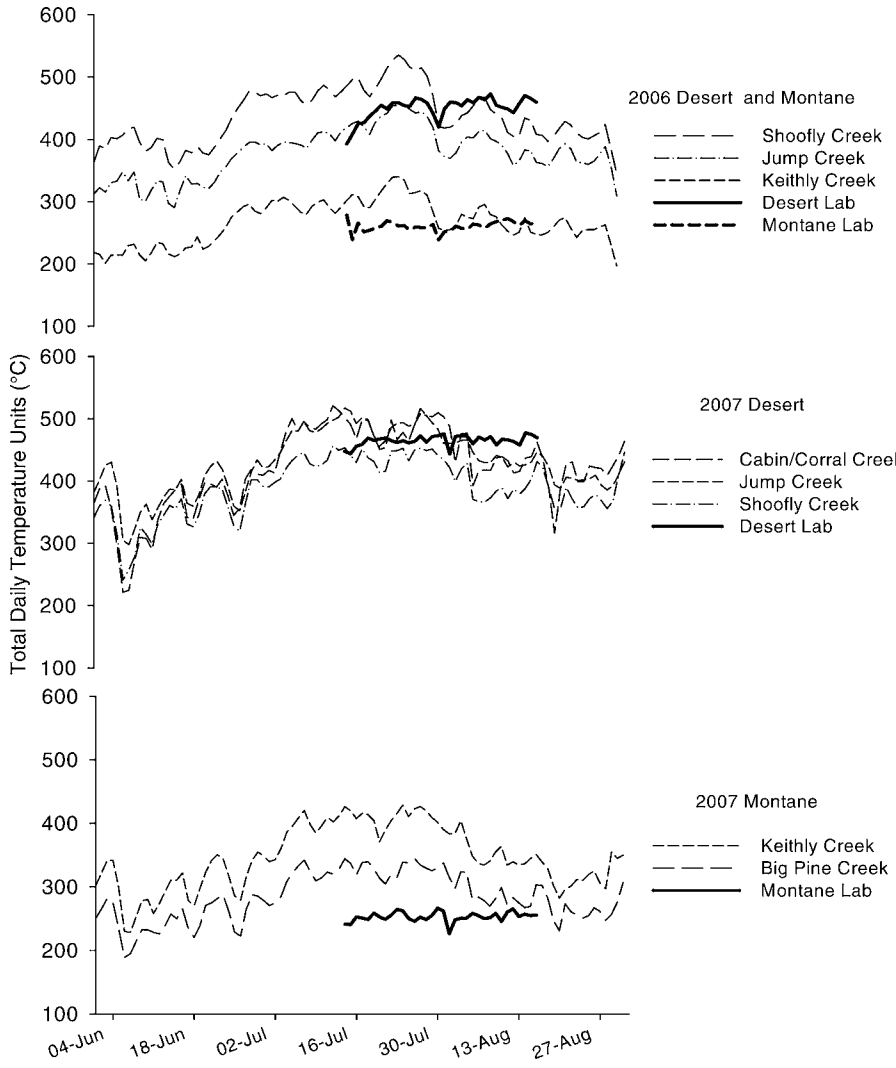

FIGURE 2.—Total daily temperature units for June, July, and August of 2006 and 2007 in desert and montane source streams and laboratory treatments with simulated desert and montane diel temperatures.

hatchery fish in montane cycles due to a limited number of tanks (Table 1).

Test of Extreme Desert Cycles

To test the thermal maxima of pre-exposed selected fish stocks in the second year of the study, we conducted a short 5-d experiment (20–24 August 2007) with a portion of the fish (~20 fish per tank) from six stocks after the 35-d exposure trials. In these trials, we increased the upper daily temperature from the previous trial's upper limit (26°C) by approximately 1°C each day. Fish were held in two replicate tanks for each stock. The temperature cycles recorded for the 5 d were: 18–25.5°C, 18–27.5°C, 18–28.2°C, 18–29.5°C, and 18–30.3°C. During the test, tanks were inspected frequently for mortalities and the time of mortality was recorded. We weighed and measured each dead fish. We recorded water temperatures and fed fish once daily using procedures detailed previously.

Sampling and Laboratory Analyses

During both 35-d studies, we removed samples of 10–15 fish from each tank for laboratory analyses at the beginning, middle, and end of the trial. We placed sampled fish immediately into aerated water with 200 mg/L buffered tricaine methanesulfonate (Finquel, Redmond, Washington) for euthanasia. We weighed

TABLE 1—Growth, feed consumption, feed efficiency, and feed conversion ratio of redband trout stocks in 35-d temperature trials by stock origin, temperature treatment, tank, and year. Year 1 trials were conducted from 15 July to 18 August in 2006, year 2 trials from 14 July to 17 August 2007.

Origin of stock and location	Year	Replicate (fish/tank)	Average fish weight (g)		Average daily growth (g)	Average growth (mm)	Daily feed eaten (g) per fish	Feed efficiency	Feed conversion ratio
			Initial	Final					
Desert temperature treatment									
Desert origin									
Jump	1	1 (150)	0.55	2.01	0.041	0.63	0.057	0.71	1.41
	1	2 (150)	0.83	1.92	0.038	0.63	0.057	0.67	1.50
	2	1 (72)	0.34	1.30	0.028	0.61	0.043	0.64	1.55
	2	2 (72)	0.32	1.51	0.034	0.60	0.042	0.81	1.24
Shoofly	1	1 (80)	0.25	1.11	0.024	0.48	0.057	0.42	2.41
	1	2 (80)	0.38	1.01	0.018	0.47	0.056	0.32	3.18
	2	1 (100)	0.38	1.00	0.018	0.47	0.031	0.56	1.77
	2	2 (100)	0.35	0.91	0.016	0.39	0.031	0.51	1.96
Cabin–Corral	2	1 (42)	0.38	1.63	0.036	0.67	0.075	0.48	2.08
	2	2 (42)	0.39	1.19	0.023	0.54	0.067	0.34	2.95
Montane origin									
Keithly	1	1 (150)	0.52	1.49	0.027	0.55	0.062	0.43	2.30
	1	2 (150)	0.49	1.35	0.024	0.51	0.055	0.43	2.33
	2	1 (41)	0.47	1.67	0.034	0.62	0.075	0.46	2.19
	2	2 (41)	0.57	1.46	0.025	0.52	0.070	0.36	2.75
Big Pine	2	1 (60)	0.31	1.32	0.029	0.57	0.054	0.53	1.89
	2	2 (60)	0.30	1.29	0.028	0.55	0.054	0.53	1.89
Hatchery origin									
Hayspur	1	1 (150)	0.57	3.74	0.088	1.09	0.123	0.71	1.40
	1	2 (150)	0.57	3.74	0.088	0.92	0.103	0.85	1.17
	2	1 (100)	0.68	4.01	0.095	0.97	0.088	0.92	1.09
	2	2 (100)	0.57	3.74	0.091	0.89	0.097	0.94	1.07
Montane temperature treatment									
Desert origin									
Jump	1	1 (150)	0.51	2.40	0.053	0.75	0.054	0.98	1.02
	1	2 (150)	0.59	2.29	0.047	0.78	0.055	0.86	1.17
	2	1 (72)	0.31	1.17	0.024	0.54	0.043	0.57	1.76
	2	2 (72)	0.31	1.19	0.025	0.58	0.040	0.64	1.57
Shoofly	1	1 (80)	0.30	1.11	0.023	0.53	0.055	0.41	2.43
	1	2 (80)	0.33	1.43	0.031	0.64	0.072	0.43	2.35
	2	1 (100)	0.37	0.71	0.010	0.40	0.033	0.29	3.43
	2	2 (100)	0.33	0.89	0.016	0.43	0.030	0.53	1.88
Montane origin									
Keithly	1	1 (150)	0.80	1.46	0.026	0.57	0.056	0.46	2.16
	1	2 (150)	0.53	1.71	0.033	0.66	0.049	0.67	1.49
	2	1 (41)	0.54	1.45	0.026	0.62	0.076	0.34	2.91
	2	2 (41)	0.57	1.65	0.031	0.61	0.070	0.44	2.26
Big Pine	2	1 (60)	0.26	1.10	0.024	0.62	0.054	0.44	2.27
	2	2 (60)	0.33	1.16	0.024	0.55	0.051	0.47	2.14
Hatchery origin									
Hayspur	1	1 (150)	0.53	3.52	0.079	0.88	0.079	0.99	1.01
	1	2 (150)	0.57	3.36	0.077	0.88	0.079	0.98	1.02

(0.01 g) and measured (1.0 mm) each fish, removed white muscle from the dorsum, and opened the peritoneal cavity to remove the liver. Tissues were stored at −80°C for later analysis of heat-shock protein 70 (hsp70). At the middle and end of the study in 2006 and at the beginning and end of the study in 2007, 5–10 fish from each tank were euthanized and stored at −30°C for proximate analysis (Selong et al. 2001). At the end of the study, we removed and euthanized all but 10 fish per tank for sampling purposes. Sampled fish were weighed and measured individually. Blood was collected within minutes of euthanization from 5 to 10 fish per tank by caudal severance and blood cells were separated from plasma by centrifugation. The plasma was representatively pooled (equal amount per fish) by tank and the samples were stored at −80°C for later determination of plasma cortisol in duplicate by radioimmunoassay (Redding et al. 1984; Barton et al. 2002).

Heat shock proteins.—Samples of muscle tissue from each fish at each sampling interval were quantified for hsp70. A pooled sample of liver tissues from each tank was analyzed because of the small size of livers. We extracted and identified proteins with Western Blotting using a modified version of the techniques described by Werner et al. (2001). We

quantified the protein by scanning the blot membranes to achieve a digital image and then quantified the density of the 70-kD blot with ImageJ software (Rasband 1997). For each sample, we calculated a ratio of the density of each hsp 70 blot to the density of a known quantity of human standard on that gel.

Growth, feed efficiency, and proximate analysis.—Changes in weight and length of fish sampled from each tank were calculated for the testing interval, and an average daily change was estimated {[(end weight − start weight)/number of fish]/35 d}. A feed efficiency [change in wet weight (g)/feed consumed (g)] was also calculated for each tank.

We used pooled samples of fish (5–7 per tank) to estimate the proximate constituents of test fish. Fish carcasses from each tank were weighed (0.001 g) and dried in a forced draft oven at 80°C for 18 h to obtain the proportion of dry tissue weight. We ground dry tissues in a coffee grinder and incinerated a portion of the tissue pool in a muffle furnace at 150°C for 50 min, 250°C for 50 min, 350°C for 50 min, and 500°C for 4 h. Ashed samples were cooled and weighed. We calculated the percent dry matter, ash free dry weight, and organic matter for each pooled sample from each tank. A portion of dried tissue from each tank that was not incinerated was weighed and sent frozen to the Hagerman Fish Culture Experiment Station in Hagerman, Idaho, where crude lipid concentration was determined by petroleum ether extraction (method 960.39; AOAC 2000) with a Goldfisch machine (Labconco, Kansas City, Missouri).

Once samples were analyzed for lipid and protein content, the lipid and protein gains were calculated for each tank by subtracting the initial proportions of lipid and protein per fish for a group from the final lipid and protein proportions per fish. Using feed consumption for the same time frame, we calculated the equivalent lipid and protein consumption and estimated lipid and protein efficiency by dividing the average lipid and protein gain per fish (g) per tank by the average amount (g) of lipids and protein consumed over the tested time interval.

Statistical Analyses

We analyzed dependent variables of feed efficiency, change in length and weight, lipid efficiency, protein efficiency, and plasma cortisol using factorial designs that varied based on the number of stocks and the inclusion or exclusion of hatchery fish. For stocks of fish used in both years of the study we added year to the factorial design as a block. For the second year when multiple desert and montane streams were used, source was also used as a blocking variable with the following model:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + \varepsilon_{ijk},$$

where i was treatment, desert or montane; j was stock, Jump, Shoofly, Keithly, (and Cabin–Corral, Big Pine in 2007), or (Hayspur); and k was replicate one or two and τ_i, β_j, $(\tau\beta)_{ij}$, and δ_k represented the effects of temperature, stock, the temperature × stock interaction, and year or source.

To analyze variables measured over time (muscle and liver hsp70), we used a repeated-measures model, namely,

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \gamma_{k(i)} + (\beta\gamma)_{jk(i)} + \varepsilon_{ijk},$$

where i was desert or montane treatment; j was fish stock, and k was tank replicate. Model factor τ_i was temperature, β_j was the effect of stock, $(\tau\beta)_{ij}$ was temperature × stock interaction, $\gamma_{k(i)}$ was time, and $(\beta\gamma)_{jk(i)}$ was the temperature × stock × time interaction. We analyzed the model including all stocks, and then with the hatchery stock omitted.

To test wild stocks that were obtained over both years, we used a multivariate model to evaluate the nonrepeated measures and the canonical structure to determine which variables were affecting the differences, namely,

$$X_{lkr} = \mu + \tau_l + \beta_k + \gamma_{lk} + \varepsilon_{lkr},$$

where μ represents the measure; τ_l the effect of stock; β_k temperature treatment, and γ_{lk} the stock × temperature treatment interaction. All statistical analyses were performed in SAS 9.0 (SAS Institute 2002), and we report P-values testing the null hypotheses.

Results

Laboratory versus Field Desert and Montane Diel Cycles

Our field-measured temperatures in source streams varied between the 2 years because of differences in snow pack and runoff. In 2006, the total hourly computed temperature units per day in the 35-d simulations were similar in intensity and duration to those observed in desert and montane source streams. In 2007, a year with less winter snow pack, we found that total daily temperature units in the laboratory montane simulation were lower than temperature units measured in the field setting (Figure 2).

Tests of Hatchery Introgression

During fish sampling we collected genetic samples from stocks to test for potential introgression with hatchery fish. Although there were no records of fish stocking in Jump Creek, the desert stock from Jump

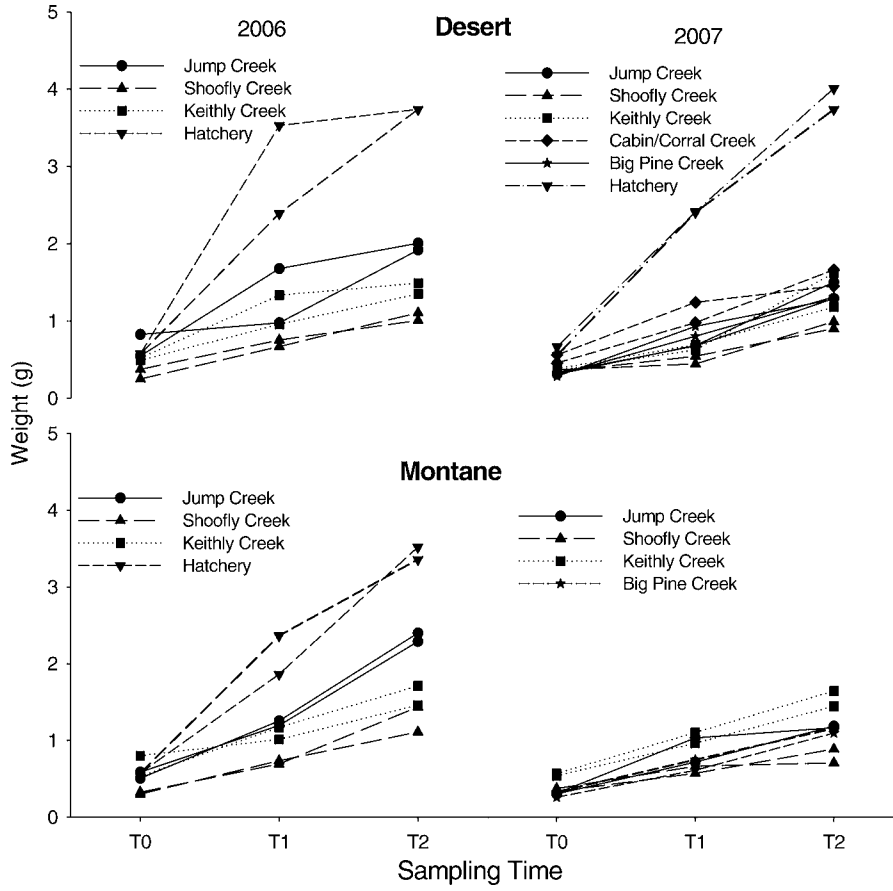

FIGURE 3.—Growth in weight of redband trout from wild and hatchery stocks at the beginning (T0), middle (T1), and end (T2) of the 35-d treatments in 2006 and 2007 by simulated desert and montane temperature treatment.

Creek showed some hatchery rainbow trout introgression. Tests of Shoofly and Keithly Creek stocks showed no introgression and the stocks from Big Pine and Cabin–Corral creeks have not been tested (C. Kozfkay and M. Campbell, Idaho Department of Fish and Game, unpublished data).

Desert and Montane Diel Cycle Experiments

Survival and growth.—We observed high survival and no differences between desert and montane stocks in 35-d temperature cycles. Mortality in the desert simulations was less than 1.5%. In both years, during the first week of desert simulations, a portion of fish (<10) had impaired equilibrium when temperatures reached daily maxima; however, most fish regained equilibrium when temperatures were lowered that day and the fish survived subsequent high temperatures. In both years, the fish from the hatchery stock grew fastest, averaging more than 0.8 mm/d in both montane and desert treatments (Table 1). Among the wild stocks, growth rates varied, and in the first year both weight ($P = 0.008$) and length ($P = 0.004$) were significantly different among all three stocks. The changes in both weight and length were greater in the montane treatments than in the desert treatments ($P = 0.027$ and $P = 0.004$, respectively; Figure 3). In 2007, we measured significant differences in weights and lengths across all stocks (weight, $P = 0.010$; length, $P = 0.003$), but we did not detect differences attributed to the temperature treatment.

When we analyzed fish weight and length with stream sources as a blocking variable in 2007, we found significantly faster mean daily increases of both weight ($P = 0.050$) and length ($P = 0.036$) in montane source populations. We found that growth rates (change in weight and length) of stocks from Jump, Shoofly, and Keithly creeks that were tested in both years were significantly different among the three stocks, the treatment, and between the years (Table 1).

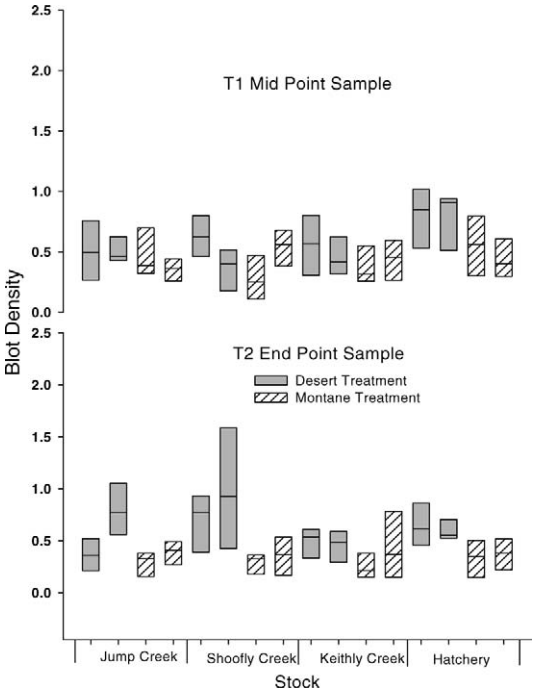

FIGURE 4.—Box plots of the densities of white muscle heat shock protein 70 in redband trout sampled from the middle (T1) and end (T2) of the 35-d temperature trial, by desert and montane treatment in 2006. Five fish were sampled from each tank. Boxes represent the entire data distribution; the horizontal lines within the boxes the medians.

Feed, protein, and lipid efficiency.—In 2006, we found Hayspur hatchery and Jump Creek stocks were the most efficient in feed conversion and the Shoofly Creek stock was the least efficient (Table 1). Across both temperatures, feed efficiencies were significantly different among stocks. Fish stocks held in montane treatments were more efficient than these same stocks held in desert treatments with and without inclusion of hatchery fish ($P = 0.003$ and $P \leq 0.020$, respectively). In the second year of tests, fish stocks held in the desert treatments were more efficient in feed conversion than those in montane treatments. Feed efficiencies were significantly different among the stocks ($P = 0.001$) and between the treatments ($P = 0.065$) for all stocks with or without hatchery fish ($P = 0.012$ and $P = 0.081$, respectively). When we tested the same three wild stocks over 2 years, using year as a blocking variable, we found feed efficiency was significantly different among the three stocks but not between the years.

In 2006 tests, hatchery fish had the highest lipid efficiency and lipid efficiencies across treatments appeared to be somewhat different among the stocks ($P = 0.069$). We found no differences between lipid or protein efficiencies between fish held in desert and montane treatments, nor did we detect differences among the wild stocks. In 2007, hatchery fish again had the highest lipid and protein efficiency. Lipid and protein efficiencies were significantly different among all stocks ($P = 0.005$ and $P = 0.012$, respectively) but not between desert and montane temperature treatments. We found lipid efficiencies were significantly different between the years when we analyzed the three wild stocks tested both years.

Plasma cortisol.—Plasma cortisol concentrations were low for all test fish in both years: less than 30 ng/mL in 2006 and less than 20 ng/mL in 2007. In 2006, we detected a significant temperature treatment × stock interaction, created by elevated plasma cortisol levels in Shoofly Creek fish held in montane treatments. Cortisol concentrations were significantly different among all stocks ($P = 0.017$) and among wild stocks ($P = 0.007$). We found no differences in plasma cortisol attributed to stock or temperature treatment in 2007; however, plasma cortisol concentrations of the three stocks evaluated in both years were significantly different between the years and among stocks.

White muscle and liver hsp70.—In 2006, fish from Jump Creek and Shoofly Creek stocks held in the desert simulation had the highest elevation of white muscle hsp70 at the end of the trials (Figure 4). We found a significant increase in muscle hsp70 levels over time ($P < 0.001$) among all fish and differences were attributed to the elevation of heat-shock proteins in fish from desert treatments at day 17 ($P = 0.014$) and day 35 ($P = 0.029$) among wild stocks. As with muscle tissues, liver hsp70 levels were also elevated in fish stocks maintained in desert treatments over fish held in the montane treatments, and hsp70 levels increased over time ($P = 0.015$).

In the second year, we found muscle hsp70 increased over time of exposure ($P < 0.001$), but quantities did not differ among stocks. When results were evaluated across sampling times among wild stocks, hsp70 was significantly elevated in desert treatments at the end of the trials ($P = 0.004$) but not in the middle samples (Figure 5). The quantity of liver hsp70 increased significantly over time within stocks held in desert temperatures ($P < 0.001$). As with muscle samples, we detected no significant differences in liver hsp70 attributed to fish stock. Testing across liver and muscle samples for all source populations revealed no significant difference in hsp70 attributed to origin of fish from desert versus montane origin.

Multivariate analysis of combined growth and stress metrics.—Using multivariate tests we identified significant differences among the three stocks tested both years (Wilk's lambda, $P < 0.001$) but not between the

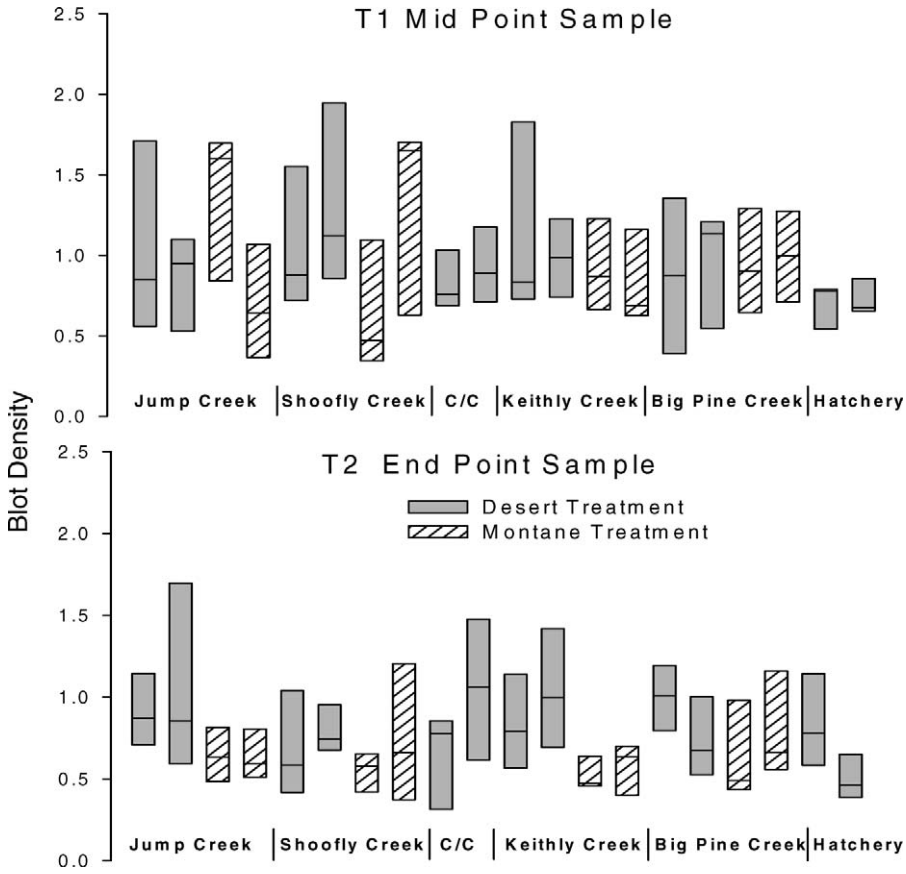

FIGURE 5.—Box plots of the densities of white muscle heat shock protein 70 in redband trout sampled from the middle (T1) and end (T2) of the 35-d temperature trial, by desert and montane treatment in 2007. See Figure 4 for additional details.

two temperature treatments. We found the change in weight ($P = 0.005$), length ($P = 0.0026$), feed efficiency ($P < 0.001$), and plasma cortisol levels ($P = 0.004$) were all significantly different among the three stocks. Using the canonical structure, we identified feed efficiency as the main driver of the significant differences in stocks.

Test in Extreme Temperature Cycles

When fish already exposed to desert conditions were placed in an incrementally increasing temperature cycle (Figure 6) all fish died in the fifth day when the maximum diel temperature reached just above 30°C. Fish from the hatchery source populations showed the earliest mortality at day 2, and by the third day nearly half of the test hatchery fish were dead. Among the wild source populations, the Jump Creek stock showed the earliest mortality with four fish (20%) dying on day 3, seven fish (44%) on day 4, and the remaining nine fish (100%) on day 5. Fish from the Keithly Creek stock did not start dying until day 4 of the trials when eight fish (53%) died; the remaining seven fish (100%) died on day 5. Fish from the remaining three wild stocks; Shoofly, Big Pine, and Cabin–Corral did not show any mortality until day 5 of the trials when all the fish from these populations died after being exposed to temperatures in excess of 30°C.

Discussion

We tested two year-classes of age-0 redband trout from both desert and montane origin stocks in a laboratory simulation of desert and montane diel temperatures. Regardless of stock origin, nearly all redband trout in the 35-d desert simulation survived a daily exposure to thermal maxima of 26°C. All stocks, including hatchery fish, were resilient to the thermal stress of the diel cycles. We observed considerable variation in the ranking of growth and stress metrics in our study with no strong trend associated with geographical fish stock source.

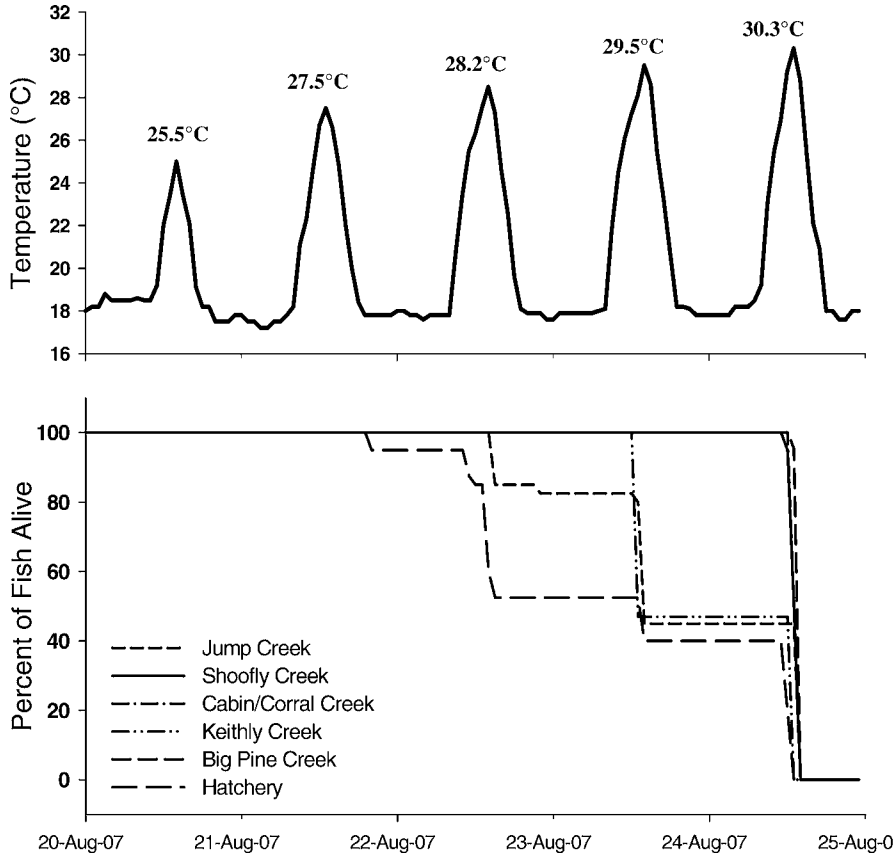

FIGURE 6.—The upper panel shows the temperature cycles in the 5-d extended diel temperature tests; the daily maximum temperatures are indicated above the peaks. The lower panel shows survival curves for each stock of fish tested by date.

Our observations of temperature tolerance and survival in desert summer conditions are in line with results reported by other investigators that show that thermal tolerance in salmonids increases when fish are exposed to high temperatures for short periods of time and provided time to recover at cooler temperatures (Dunham et al. 2003; Johnstone and Rahel 2003; Wehrly et al. 2007). Bonneville cutthroat trout *O. clarkii utah* survived exposure to cycling temperatures of 16–26°C for 7 d, even though the 7-d upper incipient lethal temperature for Bonneville cutthroat trout was estimated at 24.2°C (Johnstone and Rahel 2003). Schrank et al. (2003) found radio-tagged Bonneville cutthroat trout used stream habitat with diel maxima of 26–27°C during July and August. Dickerson and Vinyard (1999) found that groups of Lahontan cutthroat trout *O. clarkii henshawi* had high mortality rates in constant temperatures of 26°C for 7 d, but fish experienced no mortality over 7 d when temperatures were fluctuated daily from 20°C to 26°C. Numerous studies report the upper critical temperatures for rainbow trout from 26.9°C to 29.8°C depending on acclimation temperature (Lee and Rinne 1980; Currie et al. 1998; Beitinger et al. 2000).

All stocks of fish in our studies demonstrated tolerance to repeated daily high temperatures that approached the upper critical maximum because they were able to recover for many hours each day at cooler water temperatures. Fish that lost equilibrium during the high temperature were observed regaining equilibrium when temperatures dropped. Our calculations of total daily temperature units (24 hourly temperatures per day) ranged from 375 to 475 for the desert treatment, and were equivalent in units to constant temperatures of 15.6–19.8°C, which was well below upper critical temperatures for rainbow trout.

The presence of thermal refugia created from subsurface flows or stratification of low flowing pools may exist in some of these desert stream populations (Nielsen et al. 1994; Ebersole et al. 2003). Cool water refugia often serve as important areas for physiological recovery when low stream flow and excessive heating

occurs in the summer months and fish are observed congregating near them (Breau et al. 2007). While temperature studies that evaluate critical thermal maxima (CTM), incipient lethal temperature (ILT), or chronic lethal maxima (CLM) are useful to understand fish physiology, these studies rarely reflect the natural environments in streams. Bevelhimer and Bennett (2000) noted that most standard temperature criteria for managers were based on tests of CTM or ILT, and these criteria failed to consider chronic effects, and did not account for time for physiological recovery during periods of lowered temperatures. Our studies provide evidence that survival in elevated water temperatures is linked to the time spent at both elevated and lower baseline water temperatures.

Factors other than peak temperatures affect the survival of fish in elevated temperatures. Galbreath et al. (2004) reported variation among thermal maxima in rainbow trout, brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* probably were related to heating rate, water chemistry, and specific characteristics of the fish tested. Dickerson and Vinyard (1999) reported that Lahontan cutthroat trout exposed to fluctuating temperatures from 20°C to 26°C did not grow as well as control fish kept at 13°C or 20°C. Hokanson et al. (1977) concluded that the maximum temperature in which rainbow trout could maintain weight for 40 d was a constant 23°C, or a temperature fluctuating between 17°C and 25°C. Wurtsbaugh and Davis (1977) reported fish decreased growth at higher water temperatures if rations were constant. However, if fish were provided more food, growth was maximized at 17°C. Schill (2009) reported otoliths from age-0 redband trout from two Idaho desert streams showed two translucent otolith bands during their first year of life, probably associated with growth cessation induced by the cycling warm summer temperatures.

The redband trout stocks we tested showed surprisingly equivocal feeding efficiency in the simulated desert and montane environments. However, in our studies we supplied supplemental compressed air to all tanks and constant water flows. In the natural desert setting, flows could be stagnant with higher fish density in pools. Our montane treatments were probably cooler than optimal conversion temperatures for any stock, but the metabolic costs of maintenance at the elevated desert temperatures probably reduced the conversion efficiency. In addition, all fish were provided food in excess and did not have to forage. Curiously, the hatchery stocks were more efficient than wild stocks with highest conversions reported in fish from desert temperatures over those from montane temperatures during the year both temperature regimes were tested. The hatchery stocks exhibited different feeding behavior in the tanks, rising in the water column to feed, whereas wild fish were more secretive and remained near the bottom of the tank even when feeding. Other studies support more consistent growth rates and different behavior in hatchery stocks over wild fish (Kallio-Nyberg and Koljonen 1997; Reinhardt 2001; Fleming et al. 2002; Weber and Fausch 2003).

We found higher expression of heat-shock proteins in muscle and liver tissues from redband trout stocks held in desert conditions over the same source stocks held in montane temperatures. The increased elevation of heat-shock proteins in desert simulations is in line with observations by others that report heat-shock proteins elevate in response to thermal stress (Werner et al. 2005, 2006). The cellular stress response protects organisms from damage resulting from exposure to a wide variety of stressors including heat stress (Kllemade and Mothersill 2001), and the heat-shock proteins are the most important cellular defenses to chaperone proteins and to prevent and repair the damaging effects of high temperature (Feder 1999; Barton et al. 2002; Werner et al. 2006). Gedamu et al. (1983) found that continuous exposure of Chinook salmon *O. tshawytscha* embryo cells to elevated incubation temperatures of 24°C induced a transient expression of heat shock or stress proteins, whereas maintenance of the cells at a higher incubation temperature of up to 28°C resulted in continuous synthesis of these stress proteins. This in vitro translation suggests that the temperature-dependent temporal pattern of stress-protein synthesis is correlated with the levels of stress-protein mRNA. Expression of hsp70 is an index of the cellular stress response, and stress-related endocrine components will be related to the organism's ability to tolerate physiological stress (Iwama et al. 1998, 1999). Because the stress response has primary, secondary, and tertiary pathways, past thermal history has a significant effect on the patterns of expression of heat shock protein genes (Basu et al. 2002; Hofmann 2005). Although desert redband trout stocks have probably been isolated in environments where elevated water temperatures are common, the potential to respond to thermal stress and express hsp70 appears to be retained in all redband trout stocks.

Curiously, none of the fish stocks in our study showed a high elevation in concentrations of plasma cortisol. Because of small fish size, we collected blood samples only at the end of the 35-d trials after fish had been exposed to repeated diel fluctuations. Perhaps fish had habituated to repeated thermal stress. Moreover, none of the fish in our study were exercised, and disturbances were limited to feeding each day. Brown trout habituated to daily cyclical low flows after 4 d of

repeated stress had plasma cortisol levels that were no longer elevated above levels of control fish (Flodmark et al. 2002). In our trials, the gradual onset of the thermal stressor and routine recovery times during nighttime may have resulted in habituation or compensation.

Any conclusions about long-term survival of fish exposed to elevated temperatures must be cautioned by the results of our short-term (acute) exposure of fish previously stressed for 35 d. In this acute test, we observed complete mortality of fish from all stocks when water temperatures approached 30°C. These fish had been exposed previously to daily temperatures that reached 26°C for more than 40 d. If test fish had been provided a longer cooling period during each day for recovery, they may have tolerated these extreme temperatures.

Fish size and allometric relationships of larger-sized fish will probably alter the heat stress tolerance. Rodnick et al. (2004) found that at high water temperatures, larger redband trout (400–1,400 g) incurred higher metabolic costs and were more thermally sensitive than smaller fish (40–140 g). However, Rodnick et al. (2004) reported that the 6°C gradual increase in temperature led to a near doubling of oxygen consumption rates in small (40–140 g) redband trout. Our 35-d repeated trials tested small age-0 trout that were smaller than the sizes of smaller fish reported by Rodnick et al. (2004). Kieffer and Wakefield (2009) measured the oxygen consumption and ammonia excretion rates of Atlantic salmon *Salmo salar* exposed to elevated temperatures and reported protein use was more likely during lower temperatures after heat exposure. Future studies could explore these relationships and consider the effects on larger yearling fish, and the relationship with exercise and supplemental oxygen. However, extended rearing of wild stocks in the laboratory could lead to domesticated behavior, and thus may reduce the inference of such studies.

The redband trout in our study were exposed to diel water temperature cycles that closely mimicked the total exposure that would probably occur for populations in desert and montane streams of southwest Idaho. We conclude that both desert-adapted and montane-adapted populations of redband trout from the middle Snake River basin were able to withstand and respond to thermal stress equally, but our 5-d acute increasing temperature test conducted after a 35-d pre-exposure to cycle maxima of 26°C, showed that age-0 wild fish began to die when daily maximum temperatures reached 28°C, just 2°C above the thermal maxima of the 35-d trials. During drought conditions, some of the streams in the desert habitat of southwest Idaho have reached temperatures near or above 30°C for short intervals, and streams can reach temperatures above 25°C for many days in a row (Idaho Fish and Game, unpublished data). While we found no substantial physiological difference that we could attribute to desert and montane geographic origin, the desert-adapted redband trout remain more at risk from any further increases in water temperatures solely because they exist in an environment that approaches critical temperatures more frequently. The daily trends and cycles of water temperatures across all sites in our studies showed correlated daily cycles during the summer months within each year, and are known to correlate with air temperatures. Of great interest to further research is the effect of climate change on the length of time that these stocks and other salmonids are exposed to the maximum diel cycles and the frequency of maxima (Parmesan 2006; IPCC 2007, Rieman et al. 2007). Future research should focus on exploring fish response to increased length of exposure to diel cycles with peak temperatures at or above those tested in our study.

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