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Transactions of the American Fisheries Society
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/utaf20

Upper Thermal Tolerances of Rio Grande Cutthroat Trout under Constant and Fluctuating Temperatures

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Published online: 02 Sep 2013.

To cite this article: Matthew P. Zeigler , Stephen F. Brinkman , Colleen A. Caldwell , Andrew S. Todd , Matthew S. Recsetar & Scott A. Bonar (2013) Upper Thermal Tolerances of Rio Grande Cutthroat Trout under Constant and Fluctuating Temperatures, Transactions of the American Fisheries Society, 142:5, 1395-1405, DOI: 10.1080/00028487.2013.811104

To link to this article: http://dx.doi.org/10.1080/00028487.2013.811104

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Transactions of the American Fisheries Society 142:1395–1405, 2013
© American Fisheries Society 2013
ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2013.811104

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ARTICLE

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Abstract

The Rio Grande Cutthroat Trout *Oncorhynchus clarkii virginalis* is the southernmost subspecies of Cutthroat Trout, and as with the other subspecies, stream temperature regulates growth, reproductive success, distribution, and survival. An understanding of the upper thermal tolerance of Rio Grande Cutthroat Trout is important for developing water temperature standards and for assessing suitable habitat for reintroduction and management. Hatch success of Rio Grande Cutthroat Trout eggs was determined under static temperatures. The thermal requirements of fry and juveniles were also assessed under static and fluctuating temperature regimes using the acclimated chronic exposure method. Egg hatch success was 46–70% from 6°C to 16°C but declined significantly at 18°C and 20°C. Maximum growth of fry that were fed to satiation occurred at 15.3°C. The 30-d ultimate upper incipient lethal temperature (UUILT) was 22.6°C for fry and 21.7°C for juveniles. Survival during fluctuating temperature experiments was dependent upon the daily maximum temperature and the daily fluctuation. The upper thermal limits for Rio Grande Cutthroat Trout were lower than those of Rainbow Trout *O. mykiss* but similar to those of other Cutthroat Trout subspecies. The low UUILT of Rio Grande Cutthroat Trout relative to some salmonids may increase the risk of deleterious effects brought about by a changing climate, habitat alteration, and sympatric nonnative salmonids, which are known to outcompete Cutthroat Trout at temperatures above the species' optimal range. Daily mean water temperatures near the Rio Grande Cutthroat Trout's optimal growth temperature of 15°C would be suitable

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Received March 12, 2013; accepted May 28, 2013

for reintroduction of this subspecies. Depending on the daily temperature fluctuation, daily maximum temperatures within reintroduction streams and current habitat should remain at or below 25°C to ensure long-term persistence of a Rio Grande Cutthroat Trout population. This information will aid in establishing water quality standards to protect habitat where the subspecies currently occurs.

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Similar to other subspecies of Cutthroat Trout *Oncorhynchus clarkii*, the Rio Grande Cutthroat Trout *O. clarkii virginalis* is restricted to a small fraction (12%) of its historic range (Alves et al. 2008). Introduction of nonnative salmonids and habitat destruction have caused declines in the subspecies' distribution (Pritchard and Cowley 2006). Recently, the Rio Grande Cutthroat Trout was listed as a candidate for protection under the Endangered Species Act of 1973 (U.S. Federal Register 2008). Current management of this subspecies focuses on establishing new populations or expanding current populations to ensure future persistence. Although stream temperature plays a vital role in the distribution, establishment, and persistence of Cutthroat Trout populations (Harig and Fausch 2002; Dunham et al. 2003; de la Hoz Franco and Budy 2005), thermal requirements specific to the Rio Grande Cutthroat Trout have not been investigated. Current water temperature standards in New Mexico and Colorado were developed using laboratory-based thermal tolerance data for nonnative Cutthroat Trout subspecies (i.e., Bonneville Cutthroat Trout *O. clarkii utah*, Lahontan Cutthroat Trout *O. clarkii henshawi*, Snake River Fine-Spotted Cutthroat Trout *O. clarkii behnkei*, Westslope Cutthroat Trout *O. clarkii lewisi*, and Yellowstone Cutthroat Trout *O. clarkii bouvieri*; Todd et al. 2008). Variation—albeit small—in response to elevated temperatures among the Cutthroat Trout subspecies (Wagner et al. 2001) may mean that these current standards are not adequate to protect native Rio Grande Cutthroat Trout throughout the subspecies' range.

Thermal preferences and limits developed in laboratory settings are often used to assess the suitability of potential habitat for coldwater species and to establish water quality standards for protecting habitats (McCullough et al. 2001; Todd et al. 2008). Traditional laboratory studies assess thermal stress by manipulating both temperature and exposure time. The most widely used methods of testing upper thermal tolerances of fishes include the critical thermal maximum (CTMax) and the incipient lethal temperature (ILT). The CTMax method assesses acute thermal limits by subjecting fish to a linear and rapid increase of temperature (e.g., 0.3°C/min) from an acclimation temperature until a sublethal endpoint is reached, such as the loss of equilibrium or the onset of spasms (Beitinger et al. 2000). The ILT method evaluates the lethality of chronic temperatures by measuring survival for a predetermined amount of time (typically 7 d) after an abrupt transfer of fish from an acclimation temperature to environmentally relevant temperature treatment(s) (Bennett and Judd 1992). The median lethal temperature (LT50) is derived from survival data and is reported as the ILT for a specified exposure time and acclimation temperature (Beitinger et al. 2000). Using a range of acclimation and exposure temperatures, the upper ILT can be calculated as the most extreme temperature at which 50% of the population could survive indefinitely (Jobling 1981).

Although both methods are widely used to establish species' upper thermal limits, each has disadvantages, including the stochastic effects of two independent variables (time and temperature) in the CTMax method (Beitinger et al. 2000) and the confounding effects of handling on thermal stress in the ILT method (Bennett and Judd 1992). Both methods also fail to measure the effects of prolonged elevated temperatures that can occur for several weeks during the summer (Caissie 2006). The acclimated chronic exposure (ACE) method, a hybrid of the ILT and CTMax methods, tests the thermal limits of fishes by using more ecologically relevant conditions (Zale 1984; Selong et al. 2001; Bear et al. 2007). In the ACE method, acclimation temperatures are gradually increased by 1°C per day until test temperatures are reached, and the final test temperatures are maintained for long periods (30–60 d). Survival is recorded, and the ultimate upper ILT (UUILT; temperature that is lethal to 50% of the population) for the test period is estimated (Selong et al. 2001). The increased duration of the test enables the measurement of sublethal effects (e.g., a reduction in growth) in addition to survival (Widmer et al. 2006a; Bear et al. 2007).

Static temperature treatments are used to measure the upper thermal limits of fish in both the ACE and ILT methodologies. Laboratory and field studies indicate that if temperatures subsequently return to lower, nonlethal levels, fish can survive short-term exposure to high temperatures that would otherwise be lethal under long-term static conditions (Johnstone and Rahel 2003; Schrank et al. 2003). Daily minimum temperatures may provide a reprieve from otherwise stressful elevated temperatures experienced during diel temperature maxima (Dickerson and Vinyard 1999). The flexibility of the ACE method allows for the application of diel cyclic temperatures for long periods (>7 d); therefore, this method represents the most ecologically relevant measure of a species' temperature limits. However, ascertaining a useful metric that describes a species' thermal limits when the fish are subjected to a diel fluctuation is difficult because of the complexity of variables that describe a diel temperature cycle (i.e., phase duration, magnitude of the cycle, temperature minimum, and temperature maximum). Laboratory systems that are designed to mimic diel temperature fluctuations are also difficult to build and operate (Widmer et al. 2006b).

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In this study, a series of experiments was used to assess the upper thermal tolerance of early life stages of Rio Grande Cutthroat Trout. The objectives were to estimate the effects of temperature on the viability and hatch rate of Rio Grande Cutthroat Trout eggs and to determine the effects of static and fluctuating temperatures on survival and growth of Rio Grande Cutthroat Trout fry and juveniles. These results will directly benefit management of the subspecies through the development of water temperature standards and protective thermal limits.

METHODS

Experimental background and fish care.—A series of experiments was conducted at the Colorado Parks and Wildlife (CPW) Aquatic Toxicology Laboratory, Fort Collins, to assess the effects of temperature on hatch rates of Rio Grande Cutthroat Trout eggs and the effects of static and fluctuating temperatures on survival and growth of fry. A second experiment was conducted at the University of Arizona (UA) Environmental Research Laboratory, Tucson, to assess the effects of static and fluctuating temperatures on survival of juvenile Rio Grande Cutthroat Trout. Although the experiments were conducted in separate settings, the test conditions and methods were similar (Table 1).

Rio Grande Cutthroat Trout eggs were obtained from mature, ripe adults that were captured in Haypress Lake (Mineral County, Colorado). Eggs were stripped, fertilized, and water hardened in the field and were then transported to the CPW Aquatic Toxicology Laboratory. Upon arrival, eggs were treated for fungus with formalin at 1,600 mg/L for 15 min (Piper et al. 1952). The thermal testing facility used a flow-through system of eight head tanks in the static temperature experiment and three head tanks in the fluctuating temperature experiment, in which temperature was controlled using temperature programmers (Series 16B; Love Controls, Michigan City, Indiana) and aquarium heaters. Each test aquarium received water from the head tanks at a rate of 50 mL/s. Data loggers (Onset Computer Corp., Bourne, Massachusetts) recorded temperatures in test aquaria at 1-h intervals during the experiments. Temperatures were stable during all experiments and deviated little from set points, with an average SD of 0.3°C from the set point for each experiment. Mean daily temperatures during fluctuating temperature experiments were within 0.2°C of set points. Fry were fed soft-moist trout starter (Rangen, Inc., Buhl, Idaho) five times per day by using automatic feeders (Fish Mate, Conroe, Texas), and this diet was supplemented with live brine shrimp *Artemia* spp. nauplii (Argent Chemical Laboratories, Redmond, Washington). Feeding rates were adjusted to ensure that fry were fed in excess of satiation. Aquaria were cleaned daily to remove waste and uneaten food. Dissolved oxygen (mg/L), ammonia (as total N, mg/L), and nitrite (mg/L) were monitored in test aquaria throughout the entire length of each experiment and did not exceed allowable thresholds.

Age-0 fish were obtained from Seven Springs Hatchery (New Mexico Game and Fish, Jemez Springs) for use in the study at the UA Environmental Research Laboratory. The thermal testing facility used a recirculating water system with two head tanks maintained at 10°C and 35°C. Water from both head tanks was mixed to obtain test temperatures using computer-controlled Intellefaucets (Hass Manufacturing Company, Averill Park, New York). Water was delivered to 75-L test tanks for 3 min of each half-hour at a rate of 4 L/min. A recirculating water system pumped water from test tanks through a biofilter, particle filters,

TABLE 1. Comparison of methods used in static and fluctuating temperature experiments with Rio Grande Cutthroat Trout at the Colorado Parks and Wildlife (CPW) Aquatic Toxicology Laboratory and at the University of Arizona (UA) Aquatic Ecology Laboratory.

Method	CPW	UA
	Static temperature	
Pre-test acclimation	14 d at 14°C	14 d at 14°C
Increase rate	1°C per day	1°C per day
Fish size	0.18 g (14 d post-swim-up)	2.65 g (SD = 1.13 g)
Life stage	Fry	Juvenile
Density	0.47 g/L	1.13 g/L
Test temperatures	10, 12, 14, 16, 18, 20, 22, 24, 26°C	17, 19, 22, 24, 26, 28°C
Experiment duration	60 d	30 d
	Fluctuating temperature	
Pre-test acclimation	7 d at 20°C	14 d at 14°C
Increase rate	1°C per day	1°C per day
Fish size	0.88 g (110 d posthatch)	2.65 g (SD = 1.13 g)
Life stage	Fry	Juvenile
Density	0.97 g/L	1.13 g/L
Test temperatures	18–22°C and 15–25°C	16–22, 19–25, 17–27, 21–27, and 19–29°C
Experiment duration	30 d	30 d

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and a 390-W ultraviolet sterilizer, and then back to the two head tanks. Each test tank contained an air stone, a small powerhead pump (to continuously mix the water), and a thermocouple. The thermocouple in each test tank recorded temperature at 10-min intervals and was integrated with Labview software (National Instruments, Austin, Texas) for controlling tank temperatures. Temperatures during both static and fluctuating temperature experiments were consistent and deviated little from set points. The deviation from the set point averaged 0.4°C (range = 0.1–0.9°C) for static temperatures and 0.2°C (range = 0.0–0.3°C) for fluctuating temperatures during the experiments. Fish were fed daily to satiation using BioVita Starter (Bio-Oregon, Longview, Washington). All test tanks were cleaned daily to remove waste and uneaten food. Dissolved oxygen (mg/L), ammonia (as total N, mg/L), and nitrite (mg/L) were monitored daily throughout all test tanks for the entire experiment and did not exceed allowable thresholds.

Egg experiment.—Twenty eggs were distributed to incubation cups constructed from 1,000-μm-mesh nylon screen that was affixed to polyvinyl chloride pipe sections (2.5 × 2.5 × 7.5 cm) by use of aquarium-grade silicone adhesive. A single incubation cup was suspended in a 7.6-L glass aquarium and received 40 mL of water per minute from one of eight aerated, temperature-controlled head tanks. The initial temperature in each experimental unit was 12°C and was adjusted up or down over 24 h until the test temperature was reached. Survival and hatch rates were determined at test temperatures of 6, 8, 10, 12, 14, 16, 18, and 20°C; each test temperature was replicated three times. Data loggers (Onset) in each egg incubation cup recorded water temperatures at 1-h intervals. Egg mortality and hatching were monitored and recorded daily.

Static temperature experiments.—At the CPW Aquatic Toxicology Laboratory, 20 fry (mean weight = 0.18 g) were randomly selected and distributed into one of twenty-seven 7.6-L glass aquaria. Each aquarium received water at a rate of 50 mL/min from one of nine aerated, temperature-controlled head tanks. Treatments were 10, 12, 14, 16, 18, 20, 22, 24, and 26°C, and each treatment was replicated three times. The temperatures of the head tanks were initially set at 14°C for 14 d and then were adjusted to target temperatures at a rate of 1°C per day. Adjustments from acclimation temperatures were staggered so that all treatments achieved the target temperature on the same day. Five fry from each tank were subsampled without replacement to assess growth after target temperatures were attained on day 0, and fry were subsampled again at 20, 40, and 60 d. Subsampled fry were terminally anesthetized (Finquel, Argent Laboratories, Redmond, Washington), blotted dry with a paper towel, and weighed to the nearest 0.001 g. Subsampling without replacement reduced the cumulative effects of handling stress and minimized density-dependent effects among the experimental units. Although subsampling reduced the power to detect temperature-related mortality in the latter stages of the test, it is improbable that removal significantly affected the UUILT because the first subsampling event occurred after mortality was no longer observed in the study. Testing began in August 2010 and ended in October 2010.

At the UA Environmental Research Laboratory, 30 age-0 fish (mean weight = 2.6 g) were netted, measured (to the nearest mm TL), weighed (to the nearest 0.1 g), and placed into each of 18 randomly selected, 75-L tanks. Fish were acclimated at 14°C for a minimum of 14 d. After the acclimation period, temperature was increased at a rate of 1°C per day until experimental temperatures were reached. Temperature increases from acclimation temperatures were staggered to allow all treatments to reach their target temperatures on the same day. After temperature treatments were reached, they were maintained for 30 d. Treatments were 17, 19, 22, 24, 26, and 28°C; each treatment was replicated three times. Testing began in October 2010 and ended in December 2010.

Fluctuating temperature experiments.—At the CPW Aquatic Toxicology Laboratory, survival and growth of fry were assessed at a static temperature of 20°C and under two temperature fluctuations: ±2°C and ±5°C. Both fluctuation treatments had a daily mean temperature of 20°C (i.e., 20 ± 2°C = 18–22°C; 20 ± 5°C = 15–25°C) and were replicated three times. After acclimation to 20°C for 7 d, six groups of 21 fry (mean weight = 0.88 g) were randomly selected and distributed among six 19-L aquaria. Fry were added to test tanks at 1200 hours on the rising limb of the fluctuation cycle when test temperatures reached 20°C. Water from temperature-controlled head tanks was delivered to test aquaria at a rate of 90 mL/min. The minimum temperature of the fluctuation occurred at 0600 hours, and maximum temperature of the fluctuation occurred at 1800 hours. Seven fry from each tank were subsampled after 10, 20, and 30 d. Subsampled fry were euthanized, blotted dry with a paper towel, and weighed to the nearest 0.001 g.

Survival of juveniles exposed to ±3°C and ±5°C fluctuations was assessed at the UA Environmental Research Laboratory. Fluctuation treatments had daily mean temperatures of 19, 22, and 24°C; however, the 19°C trials included only a ±3°C fluctuation because of the cooling limitations of the system. The five temperature treatments were each replicated three times. At the start of the experiment, 30 fish (mean weight = 2.65 g) were randomly selected, measured (to the nearest mm TL), weighed (to the nearest 1.0 g), and placed into one of 15 randomly selected, 75-L tanks. Fish were acclimated to 14°C for a minimum of 14 d. Temperatures in test tanks were increased at a rate of 1°C per day until the daily mean of each treatment (19, 22, or 24°C) was reached. Once the daily mean was reached, temperature fluctuations were initiated. The minimum temperature occurred at 0200 hours, and the maximum temperature occurred at 1400 hours. Testing began in October 2010 and ended in December 2010.

Statistical analysis.—Egg mortality and hatch rates were arcsine–square root transformed and analyzed with ANOVA. When statistical differences were observed, means from the different temperature treatments were compared by using Tukey's test ($\alpha = 0.05$). Mean growth rate ($\mathrm{mg \cdot fish^{-1} \cdot d^{-1}}$) in each

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aquarium was determined by using linear regression of mean fry weight as a function of time. Growth rates were plotted against mean temperature, and a second-order polynomial regression line was fitted to the data to determine the temperature of maximum growth. The UUILT was calculated as the LT50 (temperature considered lethal to 50% of the fish) at the end of each experiment by using the trimmed Spearman–Karber method (Hamilton et al. 1977).

RESULTS

Egg Experiment

Hatch success of Rio Grande Cutthroat Trout eggs was 46–70% at test temperatures of 6–16°C but was significantly reduced (<22%) at 18°C and 20°C (Figure 1; $F_{7,16} = 27.3$; $P < 0.0001$). Only 1 of 60 eggs hatched at 20°C, and the sac fry died the next day. Mean number of degree-days to hatch decreased with increasing temperature (Figure 1).

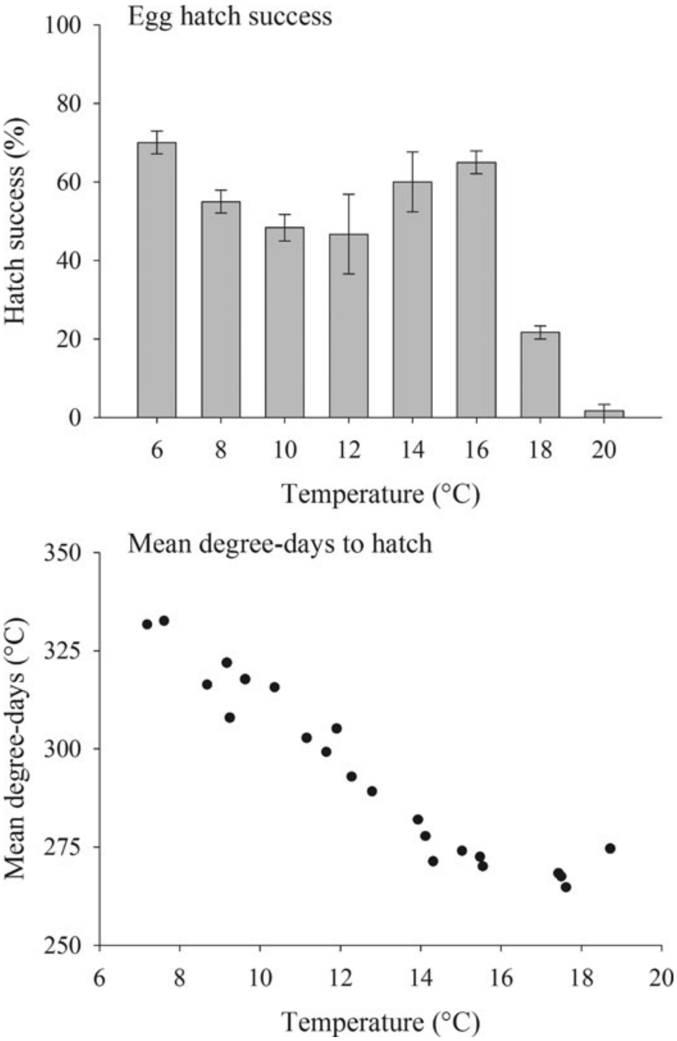

FIGURE 1. Average hatch success (%; ± SD; upper panel) and mean degree-days (°C) to hatch (lower panel) for Rio Grande Cutthroat Trout eggs in relation to temperature. Mean degree-days were calculated by summing the daily mean temperature until hatch for each egg within an experimental unit and then calculating the average for that unit.

Static Temperature Experiments

Survival of Rio Grande Cutthroat Trout fry was high (≥95%) during acclimation and did not differ among temperature treatments. Temperature-related mortality of fry occurred shortly after target temperatures were reached: all fry died within 15 d at 24°C and within 5 d at 26°C (Figure 2). During the 60-d test, survival of fry held at constant temperatures (10°C and 22°C) varied from 87% to 100%. Survival of juveniles during acclimation and temperature ramping was high (100%) for all treatments except the 28°C treatment, in which all fish died before the target temperature was attained. Mortality in the static 26°C treatment began on day 1, and all juveniles died by day 4 (Figure 2). Mortality of juveniles did not begin until day 4 in

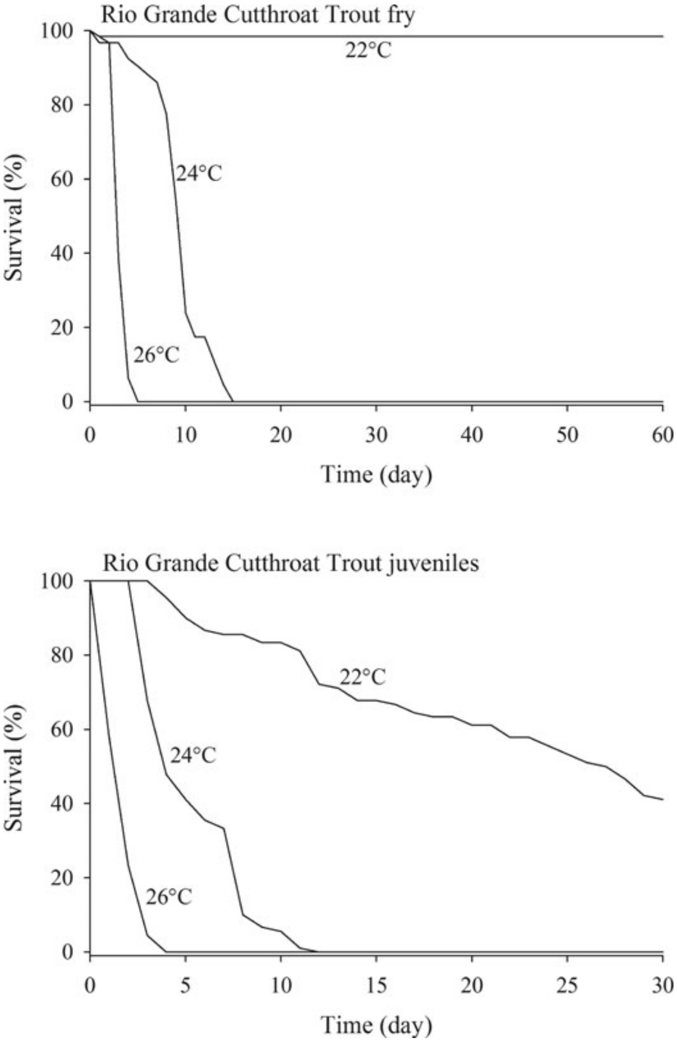

FIGURE 2. Daily mean survival (%) of Rio Grande Cutthroat Trout fry (upper panel) and juveniles (lower panel) at static temperatures of 22, 24, and 26°C. Note that the *x*-axis (time [day]) scale differs between graphs.

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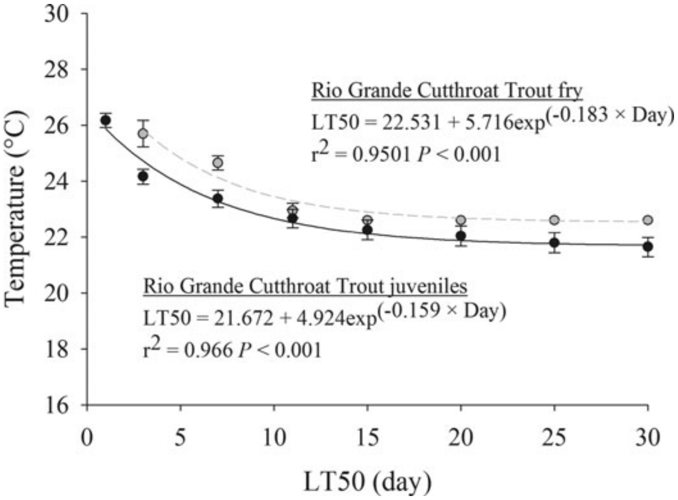

FIGURE 3. Survival (±95% confidence interval) in relation to temperature for fry (gray circles) and juveniles (black circles) of Rio Grande Cutthroat Trout. Each circle represents the temperature that was lethal to 50% of the test fish (LT50) for the given exposure time (day). Note that only the first 30 d of data for fry are depicted because no mortality occurred after day 15.

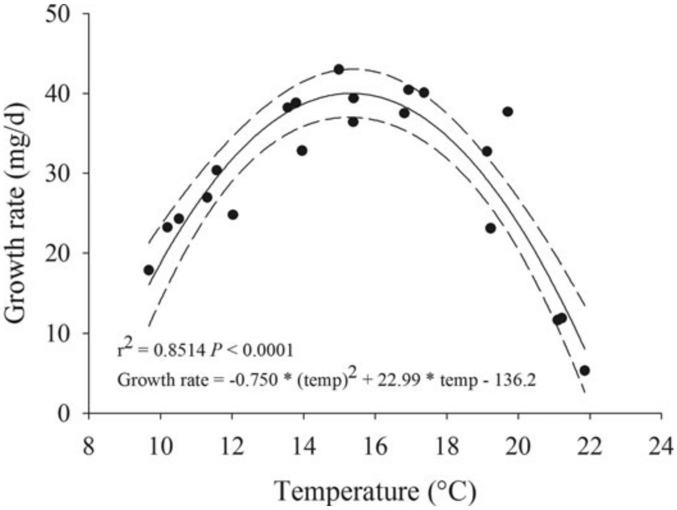

FIGURE 4. Growth rate (mg/d) of Rio Grande Cutthroat Trout fry in relation to temperature over a 60-d period. Dashed lines indicate the 95% confidence interval of the regression line.

the 22°C treatment and until day 3 in the 24°C treatment. All fish died by day 12 in the 24°C static treatment. Survival rates of juveniles in the 17°C and 19°C static temperature treatments were high (>96%) for the entire 30-d experiment.

The LT50 for fry rapidly decreased with time, from 25.7°C (95% confidence interval [CI] = 25.2–26.2°C) on day 3 to 22.6°C on day 15 (Figure 3). No mortality occurred after day 15 at any temperature. The 30-d UUILT for fry (initial mean weight = 0.18 g) was 22.6°C. The LT50 followed a similar pattern for juveniles, declining rapidly from 24.2°C (95% CI = 23.9–24.4°C) on day 3 to 22.3°C (95% CI = 21.9–22.6°C) by day 15. The 30-d UUILT for juveniles (initial mean weight = 2.65 g) was 21.7°C (95% CI = 21.3–22.0°C; Figure 3).

Growth of fry was linear over the 60-d test at constant temperatures. The R^2 for regressions of mean fry weight versus time in individual aquaria ranged from 0.91 to 0.99. Growth rate ranged from a low of 5.3 mg/d at 21.9°C to a high of 43 mg/d at 15.0°C. Growth rates increased at temperatures from 10°C to 15°C and then declined at temperatures of 18°C or higher (Figure 4). Estimated maximum growth of fry occurred at 15.3°C.

Fluctuating Temperature Experiments

Survival of Rio Grande Cutthroat Trout fry was 92% in the static 20°C treatment, 97% in the 20 ± 2°C fluctuating treatment, and 100% in the 20 ± 5°C fluctuating treatment (Figure 5). Mean growth rates were not significantly different among treatments ($F_{2,3} = 1.53$; $P = 0.28$) and averaged 60 mg·fish^{-1}·d^{-1} for the static 20°C treatment, 58 mg·fish^{-1}·d^{-1} for the 20 ± 2°C treatment, and 45 mg·fish^{-1}·d^{-1} for the 20 ± 5°C treatment.

Survival of juveniles during the acclimation and ramping period was 100%. Mortality in the 24 ± 5°C treatment began within a few minutes of reaching 29°C on the first day, and all juveniles were dead within 30 min (data not shown). In both the 22 ± 5°C and 24 ± 3°C treatments, mortality began on day 1 (Figure 5). All fish in the 22 ± 5°C treatment died by day 5, and all fish in the 24 ± 3°C treatment died by day 7 (Figure 5). Although mortality in the 22 ± 3°C treatment began on day 2, over 50% of the juveniles survived to day 17 of the experiment, and some fish survived the entire 30 d (Figure 5). Survival was 100% in the 19 ± 3°C treatment.

Sublethal Effects

In addition to decreased growth at higher temperatures, two other sublethal effects were noted. Although fry survival was not altered at 22°C, severe scoliosis occurred in 50% of the fry by 40 d and in 75% of the fry by 60 d. Three juveniles in the 22 ± 3°C treatment developed scoliosis, but they survived the entire 30-d study. Fungus *Saprolegnia* spp. developed in juveniles by day 1 of the higher temperature treatments. The incidence of *Saprolegnia* spp. was 26% (SE = 4%) in the 22 ± 3°C fluctuating treatment, 25% (SE = 4%) in the 22°C static treatment, 23% (SE = 3%) in the 24°C static treatment, and 7% (SE = 4%) in the 28°C static treatment. However, *Saprolegnia* spp. was not observed in fish that were exposed to the 17°C and 19°C static treatments or the 19 ± 3°C fluctuating treatment.

DISCUSSION

Thermal tolerances of Rio Grande Cutthroat Trout (fry and juveniles) were within the range of published values for other Cutthroat Trout subspecies. The 7-d UUILTs of Rio Grande Cutthroat Trout fry (24.7°C) and juveniles (23.4°C) were near the lower bounds of reported ranges of upper thermal limits for

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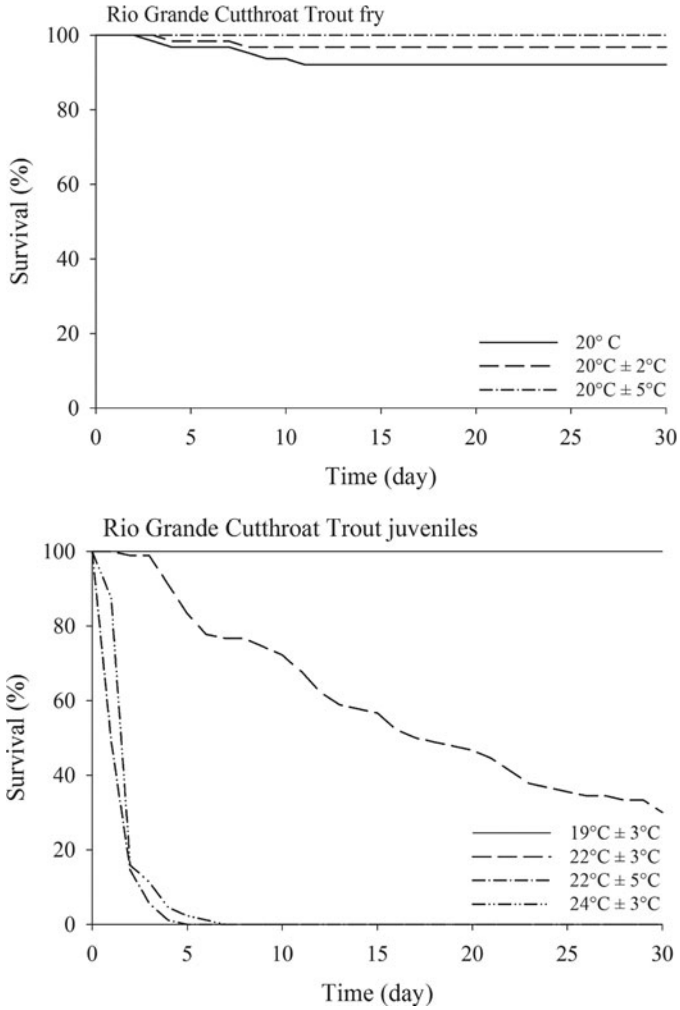

FIGURE 5. Daily mean survival (%) of Rio Grande Cutthroat Trout fry that were exposed to 20°C (static), 20 ± 2°C, and 20 ± 5°C temperature treatments for 30 d (upper panel); and mean survival of juveniles that were exposed to 19 ± 3°C, 22 ± 3°C, 22 ± 5°C, and 24 ± 3°C treatments for 30 d (lower panel).

salmonids (McCullough 1999), similar to the 7-d UUILTs of Bonneville Cutthroat Trout (24.2°C; Johnstone and Rahel 2003) and Westslope Cutthroat Trout (24.1°C; Bear et al. 2007) and the 4-d UUILT of Lahontan Cutthroat Trout (23.3–23.6°C; Vigg and Koch 1980). Of the UUILTs from these previous studies, only that of Westslope Cutthroat Trout was obtained using the ACE method, thus hindering direct comparisons among the subspecies. Compared with nonnative salmonids that occur within the subspecies' current range, the 7-d UUILT of Rio Grande Cutthroat Trout was lower than the 7-d UUILT of Rainbow Trout *O. mykiss* (26.0°C; Bear et al. 2007), which was also obtained by using the ACE method. Although the 7-d UUILT for Rio Grande Cutthroat Trout was similar to those reported for Brown Trout *Salmo trutta* (24.7°C; Elliott 1981) and Brook Trout *Salvelinus fontinalis* (24.5°C; McCormick et al. 1972), differences in acclimation procedures, fish sizes, and ages preclude direct comparisons.

The ACE method provides ecologically applicable thermal limits in comparison with traditional short-term static methods by better mimicking the thermal conditions experienced by fish in lotic and lentic environments, where high temperatures typically last for several weeks during the summer (Selong et al. 2001; Bear et al. 2007). During acclimation, the slow increase in temperature to targeted levels allows the fish to acclimate to environmentally realistic temperature changes. Extending the test duration past the traditional 7 d combines an assessment of the cumulative effects of temperature and exposure time, such as delayed mortality or other sublethal influences (i.e., decreased growth, disease, and scoliosis). For example, the 30-d UUILT was 2°C lower than the 7-d UUILT for both fry and juveniles. Westslope Cutthroat Trout and Bull Trout *Salvelinus confluentus* also exhibited delayed mortality and concomitant decreases in UUILTs at extended test durations (Selong et al. 2001; Bear et al. 2007).

The addition of treatments with fluctuating temperatures increases our understanding of the upper thermal limits of Rio Grande Cutthroat Trout by subjecting the fish to environmentally relevant conditions (Zeigler et al. 2013). We demonstrated that Rio Grande Cutthroat Trout tolerated fluctuating temperatures that were lethal under static temperature exposures. Survival of fry was not affected by the 20 ± 5°C fluctuating treatment even though temperatures exceeded the 30-d UUILT more than 40% of the time. In addition, no mortality occurred in the 19 ± 3°C treatment while daily maximum temperature exceeded the 30-d UUILT. These results are consistent with other laboratory experiments (Dickerson and Vinyard 1999; Johnstone and Rahel 2003; Widmer et al. 2006a) and field observations (Schrank et al. 2003) in which brief exposures to lethal temperatures were tolerated if fish had the opportunity to recover at lower temperatures. Juvenile Rio Grande Cutthroat Trout were unable to tolerate fluctuating temperatures when daily maxima were too high and when the duration of exposure was too long (i.e., the 22 ± 5°C, 24 ± 3°C, and 24 ± 5°C treatments).

Further investigation into the physiological effects of fluctuating temperatures on a species' upper thermal limits is needed to clarify how survival is affected by the interaction of maximum temperature and fluctuating temperature. Experimental design should focus on mimicking environmental conditions (i.e., similar fluctuations and daily maxima) with scenarios of increasing daily means and maxima to determine how increases in temperature, either anthropogenic or naturally produced, affect the species. Managers can then develop metrics to evaluate stream thermal conditions that include exposure time and the daily temperature range to assess suitable habitat. This study used daily temperature fluctuations similar to those observed in habitat that is currently occupied by Rio Grande Cutthroat Trout (Table 2; Zeigler et al. 2013). Acclimation procedures for determining thermal limits under fluctuating temperatures must also be standardized to allow for meaningful comparisons among species. One possible procedure for standardizing treatments of fluctuating temperature would be to increase the static

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TABLE 2. Information on daily temperature ranges during the summer period (July 1–September 30, 2010 and 2011) in several streams (within Colorado and New Mexico) that support populations of Rio Grande Cutthroat Trout. Daily temperature ranges in this table represent the total daily temperature fluctuation. For comparison with temperature fluctuations used in the experiment, the experimental fluctuation must be multiplied by 2. For instance, the daily range for the ± 3°C fluctuation is 6.0°C. Further information on collection of these stream temperature data is provided by Zeigler et al. (2013).

		Maximum 7-d average daily range (°C)		Minimum 7-d average daily range (°C)		Average summer daily range (°C)	
Stream	State	2010	2011	2010	2011	2010	2011
Alamosito Creek	CO	4.76	4.89	2.67	2.26	3.74	3.40
Cuates Creek	CO	3.01	3.37	1.76	1.56	2.44	2.50
Jaroso Creek	CO	5.44	6.84	3.00	2.85	4.19	4.25
North Fork Trinchera Creek	CO	6.11	7.13	3.15	3.55	4.53	5.37
Rhodes Gulch	CO	6.06	6.99	3.01	3.14	4.50	4.62
San Francisco Creek	CO	6.42	7.55	2.99	2.75	4.66	4.37
Wagon Creek	CO	6.44	8.07	3.98	3.61	5.37	5.80
West Indian Creek	CO	7.59	9.36	4.42	4.64	5.79	6.93
Little Vermejo Creek	NM	11.36	12.34	5.99	5.92	9.61	9.20
Ricardo Creek	NM	7.67	7.90	3.86	3.97	5.86	5.73
Oiser Creek	CO	9.70	10.69	5.83	4.06	7.43	6.75
Jack’s Creek	NM	5.89	6.15	2.62	2.67	4.43	4.00
Cañones Creek	NM	6.85	7.18	3.73	3.43	5.35	4.73
Cave Creek	CO	4.11	5.75	1.99	2.04	2.76	3.26
East Middle Creek	CO	7.36	8.38	4.20	2.69	5.37	4.90
Jack’s Creek	CO	10.73	7.16	5.22	2.46	8.17	4.39
Prong Creek	CO	7.88	8.44	3.21	3.30	6.02	5.45
Cabresto Creek	NM	6.39	7.80	3.53	2.88	4.94	5.01
Columbine Creek	NM	4.26	5.12	2.27	2.25	3.49	3.69
Comanche Creek	NM	12.16	13.85	5.75	5.79	9.13	10.24
Costilla Creek	NM	13.61	14.83	8.50	7.59	11.68	11.18
East Fork Costilla Creek	CO	12.10	13.48	7.85	7.13	10.47	10.30
Italianos Creek	NM	5.26	5.44	2.95	2.47	4.14	3.80
Little Costilla Creek	NM	8.13	10.13	4.00	4.70	5.72	6.96
Policarpio Creek	NM	6.70	6.09	2.98	2.72	4.80	4.60
Powderhouse Creek	NM	8.82	8.04	5.07	4.06	6.55	5.56
San Cristobal Creek	NM	3.40	4.00	1.85	1.82	2.48	2.72
Vidal Creek	NM	11.57	11.18	4.88	5.73	8.95	8.70
West Fork Costilla Creek	CO	10.59	11.22	6.80	4.82	8.30	7.71

acclimation temperature slowly (1°C per day) until the minimum or mean temperature of the fluctuation is reached. This procedure would allow fish to fully acclimate to the test temperatures, which would better mimic conditions experienced by fish in the wild. Although this would increase the length of the experiment, the importance of the acclimation procedure on temperature tolerances has been well established (Beitinger and Bennett 2000), and its standardization for fluctuating temperature experiments would be beneficial in allowing comparisons between species.

Sublethal effects other than reduced growth must be considered when assessing thermal effects on fish. Environmental stressors reduce a fish’s resistance to opportunistic pathogens (see Snieszko 1970). Not surprisingly, increased incidence of *Saprolegnia* spp. infections and scoliosis in Rio Grande Cutthroat Trout were observed at temperatures near the 30-d UUILT. Susceptibility to disease is not only dependent on the presence of a pathogen but also on the life cycle and thermal requirements of the disease. Although the coupled effects of increased temperature and disease are difficult to determine in wild salmonid populations, high temperatures and increased disease virulence were observed to cause declines in a wild Brown Trout population (Hari et al. 2006). Careful consideration must be given to the sublethal effects of temperature in wild fish populations to evaluate how climate change and increasing temperatures affect population viability.

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Ecological Implications

Responses to temperature vary among Cutthroat Trout subspecies (Wagner et al. 2001; Myrick 2008) and even among populations of the same subspecies (Vigg and Koch 1980; Drinan et al. 2012; Underwood et al. 2012). The upper thermal limits of Rio Grande Cutthroat Trout were similar to those of other Cutthroat Trout subspecies. Interestingly, the maximum growth temperature of Rio Grande Cutthroat Trout fry was 15.3°C, similar to that of Colorado River Cutthroat Trout *O. clarkii pleuriticus* (15.3–16.4°C; Brandt 2009), slightly higher than those of Yellowstone Cutthroat Trout and Snake River Cutthroat Trout (14.5–14.7°C; Myrick 2008), and higher than that of Westslope Cutthroat Trout (13.6°C; Bear et al. 2007). Differences in temperature at maximum growth indicate an apparent latitudinal pattern. The Rio Grande Cutthroat Trout has the southernmost distribution among Cutthroat Trout subspecies, and as such, this subspecies can be expected to be more warm-adapted than other subspecies. Strong support exists for local thermal adaptation within Cutthroat Trout populations (Drinan et al. 2012). The latitudinal trend suggests that subspecies of Cutthroat Trout have become thermally adapted to the temperature regimes where they reside, effectively maximizing their growth at the temperature most commonly experienced by each subspecies.

While support exists for local thermal adaptation of maximum growth, the UUILTs of Cutthroat Trout subspecies do not exhibit a similar latitudinal pattern. Adaptation to temperature for growth raises the question as to why adaptation to elevated temperatures is not manifested in thermal tolerances as well (see McCullough et al. 2009). In salmonids, for example, there appears to be little variation in upper thermal tolerances among genera (McCullough 1999). Size-mediated differences in thermal limits within a species (Recsetar et al. 2012; Underwood et al. 2012) may be related to biomechanical (surface area : volume ratio) processes.

Applicability of Laboratory Tests

Laboratory assessments of thermal limits eliminate complicating conditions, such as disease or food limitations. This study combined a series of experiments that examined the upper thermal tolerances of Rio Grande Cutthroat Trout early life stages (eggs and fry) and juveniles. Mortality of fry and juveniles was rapid at static temperatures of 24°C and 26°C. The UUILTs of the fry and juveniles tracked closely through time, whereas the UUILT of juveniles was about 1°C lower, presumably due to mortality of juveniles at 22°C, which did not occur among fry. Differences in the UUILT between the two life stages may have been caused by the sources of test fish, the observed *Saprolegnia* spp. infections in juveniles, and differences in the sizes of test fish (initial mean weights = 0.18 g versus 2.65 g). Although larger fish are considered more thermally sensitive to high water temperatures than smaller fish (Meeuwig et al. 2004; Underwood et al. 2012), an effect of size on thermal tolerance is not consistent in the literature (De Staso and Rahel 1994; Wagner et al. 2001; Recsetar et al. 2012). Differences in upper thermal tolerances due to the source of the test fish have also been observed in some species (Fields et al. 1987), although differences among salmonid species have demonstrated mixed results (see McCullough et al. 2009). Egg hatch success and timing (degree-days) of Rio Grande Cutthroat Trout were in agreement with results for other salmonids, indicating that higher temperatures decreased hatch success and hatch timing (Baird et al. 2002). Differences in thermal limits among Cutthroat Trout merit further research given the critical status of the majority of subspecies, their susceptibility to climate change (Wenger et al. 2011), and their importance in establishing cold-water temperature standards (Todd et al. 2008).

The fundamental thermal niche, defined as the range from 3°C lower to 1°C higher than the optimal growth temperature (Christie and Regier 1998), is 13.3–16.3°C for Rio Grande Cutthroat Trout. Although the fundamental niche was calculated under ideal conditions in a laboratory setting, one can expect that as temperature exceeds the thermal limits, a decrease in individual growth and a reduction in population viability will occur. Decreased food availability and the presence of nonnative fishes may shift the fundamental thermal niche to lower temperatures (Wootton 1998; Taniguchi and Nakano 2000). Coupling of laboratory findings with field observations of realized thermal niches could resolve the thermal requirements of targeted species (Huff et al. 2005). Although there is no empirical evidence describing the preferred thermal niche of Rio Grande Cutthroat Trout, historic and current distributions provide ancillary support that the subspecies' suitable temperature range is 19.0°C and below (Haak et al. 2010). This is similar to the thermal range observed in our study. Daily maximum temperatures near 25°C appear to be near the upper limits for Rio Grande Cutthroat Trout when exposed to fluctuating temperatures. Survival of fish that are exposed to daily maximum temperatures of 25°C may depend on the diel temperature range and the amount of time the fish spend at cooler temperatures during the day. Although the upper thermal limits of Rio Grande Cutthroat Trout are similar to those of other Cutthroat Trout subspecies, the low upper thermal limits of the Rio Grande Cutthroat Trout increase this subspecies' risk of deleterious effects from increased stream temperature caused by a changing climate and habitat alterations. The ability of nonnative salmonids to outcompete Cutthroat Trout at temperatures above a subspecies' optimal limit (De Staso and Rahel 1994; Bear et al. 2007) will also lead to increased negative interactions among fishes as stream temperatures increase in the future.

ACKNOWLEDGMENTS

A Crockett and P. Forsburg assisted with data collection and the care of embryos and fry for experiments conducted at the CPW Aquatic Toxicology Laboratory, Fort Collins. R. Galindo and S. Hall assisted with experiments conducted at the UA Environmental Research Laboratory. We also thank K. Patten,

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M. Sloane, and T. Jacobson (New Mexico Department of Game and Fish) for providing study fish used at the UA Environmental Research Laboratory. The study at the CPW Aquatic Toxicology Laboratory was supported in part by the U.S. Fish and Wildlife Service (Federal Aid Grant F-243). Funding for the study at the UA laboratory was provided by the U.S. Geological Survey's Science Support Partnership. Additional funding was provided by the Agriculture Experiment Station and Department of Fish, Wildlife, and Conservation Ecology at New Mexico State University. Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government. This study was performed under the auspices of New Mexico State University's Institutional Animal Care and Use Committee (Number 2009-006).

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