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Combined Effects of Temperature and High pH on Mortality and the Stress Response of Rainbow Trout after Stocking

ERIC J. WAGNER, THOMAS BOSAKOWSKI,[1] AND STEVEN INTELMANN

Utah Division of Wildlife Resources, Fisheries Experiment Station
Logan, Utah 84321, USA

Abstract.—To improve survival of stocked fish, field and laboratory tests were conducted to evaluate the survival and stress response of rainbow trout *Oncorhynchus mykiss* after exposure to waters with various combinations of high temperature and high pH. For each of four laboratory experiments, fish were transported by truck for 90 min. Fish were then put in replicate tanks for each of four treatments per experiment: (A) controls, pH 7.8, temperature 14°C; (B) control pH and high temperatures (19°C, experiment 2; 22°C, experiments 1, and 4) or low temperatures (7–9°C, experiment 3); (C) control temperature and fluctuating high pH (8.4–9.6); and (D) a combination of high or low temperature with high pH. Blood was sampled at 1.5, 3, 6, and 24 h after stocking. At 1.5 h, plasma glucose, chloride, and cortisol levels in all fish indicated a stress response from hauling and stocking. High temperatures (19°C, 22°C) alone did not produce additional changes in the stress indicators. However, high pH induced significant additional rises in glucose and cortisol levels in both high- and control-temperature tanks. At low temperatures the stress response was delayed; low temperature (7–9°C) alone produced significant elevations in glucose and cortisol compared with controls at 24 h, suggesting that cold water acted as a stressor, albeit with a delayed reaction. Cold water combined with high pH induced higher cortisol concentrations after 24 h than high pH alone. Warm temperatures combined with high pH did not synergistically affect the stress response, but they significantly increased mortality at 22°C. Mortality in the field occurred at pH levels greater than 9.3–9.4 and temperatures of 19.9–22.8°C. Diel fluctuations in pH measured in four reservoirs ranged 0.1–0.5 units. Laboratory and field tests indicated that pH values greater than 9.4 resulted in mortality, especially at higher temperatures. There was also a significant stress response to pH 9.0 or greater.

A temperature increase or decrease can be lethal to fish, depending upon various factors such as the net difference in temperatures, acclimation temperature (Brett 1956), genetics (Ihssen 1973), dissolved oxygen concentrations (Rutledge and Beitinger 1989), and water hardness (Craigie 1963). For rainbow trout *Oncorhynchus mykiss,* the upper incipient lethal temperature has been established for various acclimation temperatures, with an ultimate upper incipient lethal temperature of 27°C (Charlon et al. 1970). The critical thermal maximum reported for rainbow trout was 28–29°C (Lee and Rinne 1980). Sublethal temperature increases or decreases can be stressful as well (Wedemeyer 1973; Strange et al. 1977; Thomas et al. 1986).

An increase or decrease in pH can also be lethal (for reviews, see Doudoroff and Katz 1950; Exley and Phillips 1988). Upper and lower pH limits depend on the acclimation pH (Jordan and Lloyd 1964), aluminum concentrations (Witters 1986; Ingersoll et al. 1990), calcium concentrations (Brown et al. 1989), fish species (Doudoroff and Katz 1950; Narahara et al. 1996), and other factors. As with temperature, changes in pH that are sublethal can evoke a physiological stress response (Goss and Wood 1988; Brown et al. 1989; Whitehead and Brown 1989).

Stress response studies have generally looked at one stressor and its effect on the fish species under investigation. See Pickering (1981) and Barton and Iwama (1991) for reviews of the various stressors.

Studies that evaluate combined stressors are limited. Barton et al. (1986) and Sigismondi and Weber (1988) have studied the effects of repeated handling, both noting a cumulative effect on the physiological and behavioral response of juvenile chinook salmon *O. tshawytscha.* Pickering and Pottinger (1987) noted that elevated ammonia in combination with reduced pH significantly increased the plasma cortisol levels in response to acute confinement, whereas a combination of reduced oxygen (100% to 20% saturation), elevated free CO_2 and elevated ammonia markedly suppressed the cortisol response of both brown trout *Salmo trutta* and rainbow trout. Barton et al. (1980) found cumulative effects on the plasma cortisol response of fingerling rainbow trout due to sequential handling, confinement, transport, and stocking.

[1] Present address: Beak Consultants, 12931 Northeast 126th Place, Kirkland, Washington 98034, USA.

Multiple stressors are the norm under field conditions. Both temperature and pH are known to increase during summer months in the lacustrine waters of Utah (Utah Division of Wildlife Resources, unpublished data), potentially compromising the survival rates of stocked trout. We evaluated mortality and the stress response of rainbow trout to a combination of two environmental stressors, temperature and pH changes, in the context of a typical stocking operation. The objective of the research was to improve the survival of stocked rainbow trout by identifying water quality conditions in which survival may be compromised. The evaluation presented here is a compilation of several experiments conducted in both laboratory (preliminary experiment and wet laboratory tests) and field settings.

Methods

The laboratory experiments began with a preliminary test to examine the effects of combined high temperature (21.7°C) and high pH (9.0) on mortality and the stress response. Next, four separate laboratory tests examined the stress response and mortality of fish exposed to other combinations of high pH and temperature; the fourth test was conducted with a different strain of rainbow trout to evaluate possible strain differences. Field survival tests were conducted in reservoirs with a history of high pH to corroborate lethal limits derived from the laboratory tests. A field stress response test was also conducted at Mantua Reservoir.

Preliminary test.—Rainbow trout of the Sand Creek strain were raised from eggs at the Fisheries Experiment Station in Logan, Utah. The fish (mean weight, 58 g; mean length, 174 mm) were kept off feed for 76 h before the experiment on 24 September 1991. Total hardness and total alkalinity of the hatchery water were both 222 mg/L.

Fish from the concrete raceways were loaded with dip nets into a 623-L fiberglass transport tank supplied with oxygen. The loading density was 106.9 kg/m^3. The truck and tank were driven for 85 min to simulate a stocking trip. Upon return, 50 fish were hand counted from dip nets into each of eight raceways.

There were four treatments with two replicates per treatment. The four treatments were (1) high pH (9.0 ± 0.05) and high temperature (21.7 ± 0.3°C), (2) high temperature (21.7 ± 0.3°C), (3) high pH (9.0 ± 0.05), and (4) control (pH 7.7–7.8, temperature 16.3–16.6°C). High-pH treatments were created by adding equal amounts of sodium carbonate to each of the appropriate raceways. High-temperature treatments were created by pumping heated water from a raceway with immersion heaters to each of the appropriate raceways. The raceways (7.00 m × 0.92 m × 0.48 m) were not supplied with additional water flow during the experiment, but oxygen was delivered to each raceway through air stones.

A baseline sample of 20 fish was collected before loading by netting the fish into a bucket with tricaine methanesulfonate (MS-222) anesthetic (100 mg/L). Of the transported fish, 10 from each raceway were also netted into buckets with MS-222 1.5 h after stocking. Immediately after induction of anesthesia, blood was taken from the caudal vasculature with a heparinized syringe. The needle was removed from the syringe and the blood was gently expelled into a microcentrifuge tube kept in an ice water bath. After centrifugation for 5 min, the plasma was pipetted into another prelabeled centrifuge tube and kept frozen at −70°C until the sample was analyzed. The order of sampling was conducted so that the first 40 fish included all treatments, and the next 40 fish were sampled from the remaining replicate raceways, resulting in a 20-fish sample from each treatment.

The test temperatures and pH were recorded at the beginning and end of the 24-h exposure. The pH of each raceway was measured with an electronic digital pH meter (Orion Research, Cambridge, Massachusetts), calibrated the day of the experiment with standard buffers. Samples and standards for pH measurement were kept in a water bath (14 ± 1°C) to avoid bias due to temperature fluctuations. Temperatures were measured with a digital probe, checked for accuracy against a mercury thermometer.

Plasma cortisol was radioimmunoassayed according to the modifications made by Redding et al. (1984) to the procedure of Foster and Dunn (1974). The standards used in the radioimmunoassay were prepared from salmonid plasma stripped of endogenous steroids with activated charcoal. Plasma chloride concentrations were determined by amperometric–coulometric titration (chloridometer, Haake-Buchler Instruments, Saddle Brook, New Jersey).

Laboratory tests.—Rainbow trout of two strains were raised from eggs at the Fisheries Experiment Station in Logan, Utah. Sand Creek strain rainbow trout (mean weight, 45–65 g) were used in experiments 1 (9 February 1993), 2 (23 March 1993), and 3 (27 April 1993). Ten Sleep strain rainbow trout (mean weight, 19 g) were used in experiment

4 (8 June 1993) to test for possible strain differences in stress response. Test fish were kept off feed for at least 24 h before each experiment.

Fish from the raceways were loaded with dip nets into a 623-L fiberglass transport tank supplied with oxygen. The loading density was approximately 0.178 kg/L for each trip and 0.87 kg of noniodized salt (1.4 g/L) was added to each tankful. The truck and tank were driven around for 90 min to simulate a stocking trip. Upon return, 80 fish were hand counted from dip nets into each of eight fiberglass tanks (800 L).

Four experiments were conducted with two strains of rainbow trout. Each experiment had four treatments with two replicate tanks per treatment. The four treatments included (A) a control, (B) a high- or low-temperature group, (C) a high-pH group, and (D) a group exposed to a combination of high pH and high or low temperature. Control pH (7.8) and temperature (14°C) were the same for all treatment conditions for each experiment; treatment conditions for each experiment were as follows.

Experiment 1.—Sand Creek strain: (A) control; (B) control pH, high temperature (19–22°C); (C) high pH (9.0–9.5), control temperature; and (D) high pH (9.0–9.5), high temperature (19–22°C).

Experiment 2.—Sand Creek strain: (A) control; (B) control pH, high temperature (18–20°C); (C) high pH (8.4–9.6), control temperature; and (D) high pH (8.4–9.6), high temperature (18–20°C).

Experiment 3.—Sand Creek strain: (A) control; (B) control pH, low temperature (7–9°C); (C) high pH (8.6–9.6), control temperature; and (D) high pH (8.6–9.6), low temperature (7–9°C).

Experiment 4.—Ten Sleep strain: (A) control; (B) control pH, high temperature (20–22°C); (C) pH (8.4–9.5), control temperature; and (D) high pH (8.4–9.5), high temperature (20–22°C).

A temperature of 22°C was the maximum tested because this was the maximum temperature reported in recent stocking records of the Utah Division of Wildlife.

The pH was increased to 9.5 by adding reagent-grade sodium hydroxide to normal hatchery well water (total hardness, 222 mg/L; total alkalinity, 222 mg/L). The water was mixed in a raceway and tested to insure proper pH and temperature before being pumped into the appropriate circular tanks. Temperature was modified with immersion heaters or with a water chilling unit. The test conditions were maintained for 96 h as indicated in Figure 1. Water was exchanged twice each day in the first experiment and four times per day in the remaining experiments. About a third of the tank volume was added during each water exchange. The pH was allowed to drop overnight, simulating a diel cycle observed in some waters (Philip 1927; Reddy 1981; Wylie and Jones 1987). Ammonia was tested by the Nesslerization method (APHA et al. 1989) in each tank for each experiment and did not exceed 0.075 mg un-ionized NH_3-N/L.

Immediately before each experiment, a baseline blood sample was collected from 20 fish before loading the stocking truck. At 1.5, 3, 6, and 24 h after transport and "stocking," 10 fish from each tank were also netted into buckets with MS-222. Immediately after induction of anesthesia, blood was taken from the caudal vasculature, and the plasma was handled as described above.

The 40 fish remaining in each of the circular tanks were left for 96 h to determine the impact of water quality on delayed mortality. The test temperature and pH were recorded at the beginning of each experiment and before and after each water exchange thereafter. Temperature and pH of each tank were measured as described for the preliminary experiment.

Plasma cortisol was assayed by using the enzyme-linked immunosorbent assay (ELISA) method (Barry et al. 1993). Plasma glucose was assayed by an enzymatic (hexokinase) method and a diagnostic kit (procedure 16-UV, Sigma Diagnostics, Saint Louis, Missouri). Glucose assays were conducted in duplicate in 96-well plates (0.35-mL wells). A standard curve of 0, 100, 200, and 300 mg/dL was included on each plate by using commercial standards. The plates were read at 340 nm with an ELISA plate reader (Titertek Multiskan MCC/340 MK II, Flow Laboratories, McLean, Virginia). Plasma chloride concentrations were determined by amperometric–coulometric titration.

Field tests.—Survival in waters of high pH and high temperature was evaluated during the summers of 1993, 1994, and 1996. Selection of test waters was based on stocking records. For each site, 50 fish were put into each of two live cages (1 m in diameter by 3 m deep, 1-cm-stretch mesh of woven nylon netting). The cages were anchored so that full access to the range of depths was available to the fish and to avoid problems with bank anglers. Cage stockings at Nine Mile Reservoir, Spirit Lake, and Payson Reservoir were coordinated with a scheduled stock of the water or waters nearby, but Mantua Reservoir was stocked with fish loaded solely for the tests. The pH and tem-

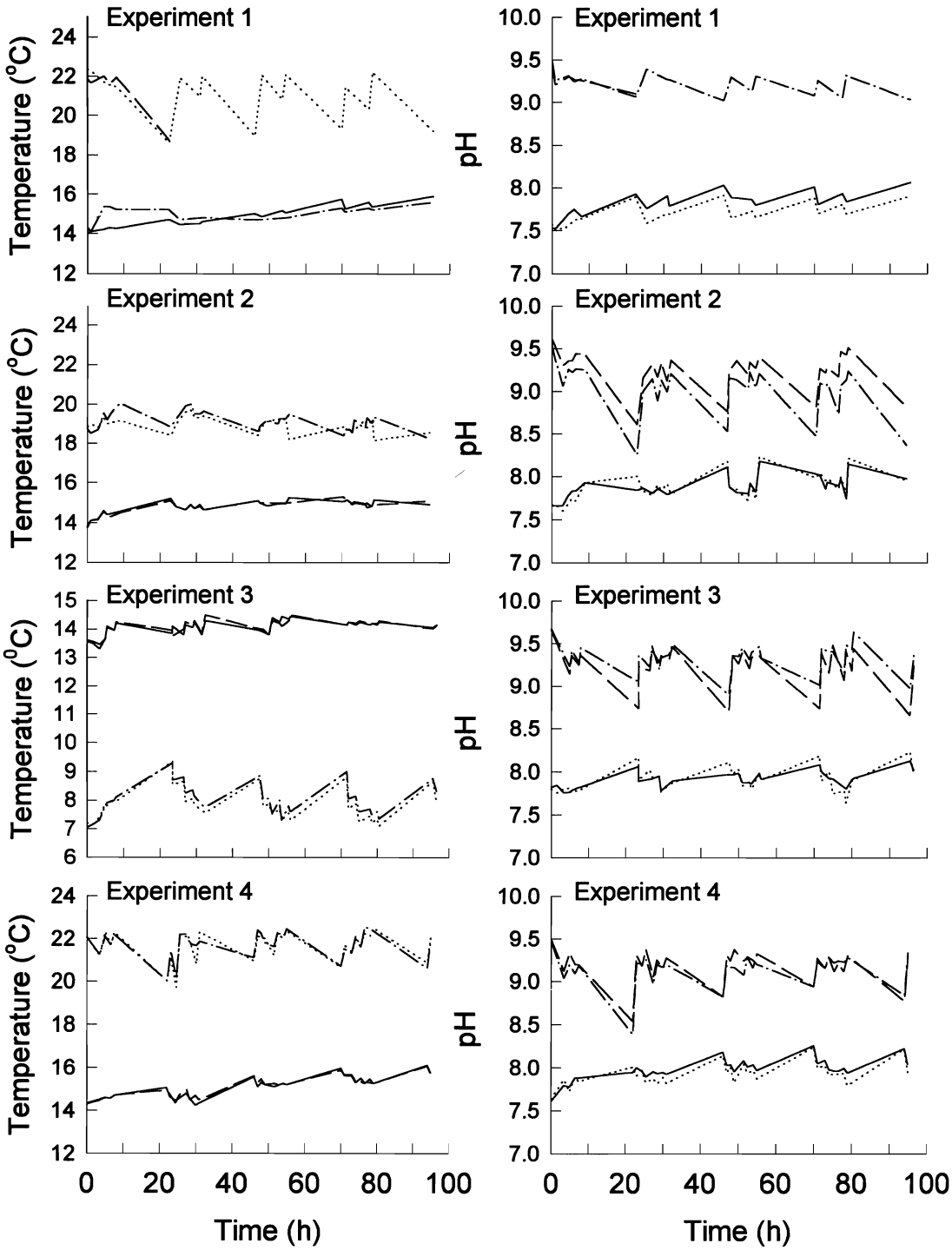

FIGURE 1.—Actual mean temperature (°C) and pH during the course of each of the four wet laboratory tests (experiments 1–4, defined in Methods), simulating natural diel fluctuations. Solid line: control treatment; dotted line: high- or low-temperature treatment; dash–dot line: high-pH treatment; and dashed line: combination treatment (high pH plus high or low temperature).

TABLE 1.—Water quality conditions and percent survival (N = 100) of rainbow trout in field tests in selected Utah waters 96 h after stocking. Ranges of water conditions are across depth and time.

Water body	Date	Hatchery conditions		Field conditions		Field survival (%)
		Temperature (°C)	pH	Temperature range (°C)	pH range	
Nine Mile Reservoir	8 Aug 1994	15.7	7.8	20.9–24.6	9.2–9.6	1.0
Mantua Reservoir	10 Aug 1993	17.1	7.5	19.3–24.3	9.2–9.6	61.1
	12 Aug 1996	9.0	7.8	19.9–25.5	9.2–9.9	0.0
	22 Aug 1994	13.9	7.6	20.2–22.0	8.5–9.1	99.0
Spirit Lake	19 Jul 1993	14.7	6.8	11.2–14.0	6.8–7.4	96.9
Payson Reservoir	11 Jul 1994	14.8	7.4	16.2–22.1	7.6–8.5	100.0

perature of the hatchery of origin for the tests is given in Table 1.

Temperature and pH were monitored every 2 h from the time of stocking until 96 h later, from sunup to sundown. It was assumed that pH would be at its maximum and mimimum during this time, so nighttime sampling was not done. Measurement of pH was performed with either a submersible probe (Hydrolab, Austin, Texas) or an Orion pH meter calibrated with standard buffers just before the test. Other water quality measurements included a one-time measurement of total hardness, total alkalinity, Secchi depth, and specific conductivity. After 96 h, mortality in the cages was determined, and the surviving fish were liberated.

At Mantua Reservoir, additional information was collected regarding the stress response on 10–14 August 1993. At 1.5, 3, 6, and 24 h after stocking, 20 rainbow trout were anesthetized, and blood was collected as described previously. The plasma was frozen and later analyzed for chloride, glucose, and cortisol concentrations. Sampling for each time period was alternated between two cages (100 fish/cage) to minimize disturbance of the fish. Control fish were stocked into identical cages in cobble-bottomed concrete ponds at the Fisheries Experiment Station and were sampled at the same times.

Description of study reservoirs in Utah.—Mantua Reservoir in Box Elder County has 224 ha of surface area, a maximum depth of 6.1 m, and an average depth of 4.3 m (UDH 1982). Secchi depth measurements at the time of the survival tests ranged from 1.1 to 1.5 m. Dissolved oxygen (DO) measurements in 1993 and 1994 indicated that the top 2–3 m were well oxygenated, but the bottom 0.5–0.8 m varied from 0.8 to 6.4 mg/L during the study. In 1996, DO was suitable for fish from top to bottom. Total hardness was 120–154 mg/L and total alkalinity was 137 mg/L.

Nine Mile Reservoir in Sanpete County has a surface area of 80 ha, mean depth of 4.6 m, and maximum depth of 9.4 m (UDH 1982). Due to drawdown, maximum depth at the time of the test was 4.0 m. Secchi depth at the time of the test was 1.2 m. Dissolved oxygen during the test was more than 4.5 mg/L in the top 3 m of the water column but was often less than this near the bottom. Total hardness (measured as $CaCO_3$) was 308 mg/L, and total alkalinity was 342 mg/L.

Payson Reservoir (also known as Big East Lake) in Utah County has a surface area of 9.3 ha and a maximum depth of 6.2 m. During the test it had a Secchi depth of 3.1 m. Dissolved oxygen was more than 5 mg/L in the first 4 m of the water column but was unsuitable for salmonids below this depth. Total hardness was 85 mg/L and total alkalinity was 103 mg/L.

Spirit Lake, Daggett County, Utah, has a surface area of 16.6 ha, maximum depth of 5.5 m, and average depth of 4 m (UDH 1982). During the test, Secchi depth was 2.5 m and DO was more than 7.0 mg/L at all depths. Total hardness was 17 mg/L and total alkalinity was 14 mg/L.

Statistical analysis.—A significance level of $P < 0.05$ was used for each test. One-way analysis of variance (ANOVA) was used to test for differences in treatment means of the preliminary test. Fisher's least-significant-difference test was used for subsequent mean comparisons (SAS 1990a).

For Mantua Reservoir stress test results, treatment means at each time period were compared statistically with the control group by the Student's t-test (Number Cruncher Statistical Software, Kaysville, Utah). If variances were not equal, an unequal-variance t-test was applied. Cortisol, glucose, and chloride results were tested for normality with the Shapiro–Wilk test (SAS 1990a). The General Linear Model (GLM) analysis of variance procedure (SAS 1990b) was used for experiment 1 because sample sizes were unequal due to mortality. The model included treatment and replicate effects and the interaction term. The remaining experiments were analyzed with a two-way ANO-

VA, blocking for replicate effects. Mean comparisons were made with Fisher's least-significance-difference test or Duncan's multiple-range test (SAS 1990b; SPSS 1993). Both the GLM and standard ANOVA analyses were done separately for each sampling time period. Replicates were treated as a random variable in both GLM and standard ANOVA tests. Tests for differences in cortisol between the baseline sample and each of the sixteen treatment–time samples were made by the Wilcoxon test (experiment 1) or a two-sample *t*-test (remaining experiments). Percent mortality data were analyzed by chi-square analysis, first testing for replicate differences. Replicates proved to be insignificantly different, so mortality data was pooled for each treatment before subsequent testing of (1) treatment differences using all treatments and (2) partial tables that included only two treatments per table (Fienberg 1980).

Results

Preliminary Test

All treatment groups had significantly lower plasma chloride levels than baseline fish 1.5 h after transport (Figure 2A). This result indicated that handling and transport stress caused a reduction in plasma chloride. However, no differences in plasma chloride were found between the various treatment groups at the time sampled in the preliminary test. This uniformity indicated that the changes in both temperature and pH used in our experiment did not induce an additional or cumulative stress response with regard to plasma chloride homeostasis by the time the fish were sampled.

Plasma cortisol levels increased significantly in all treatment groups in response to the stress of handling and transport (Figure 2B). The high-pH (9.0) group had significantly higher cortisol concentrations than the remaining treatment groups. Warm temperatures (22°C) in combination with high pH significantly reduced the cortisol response from the level resulting from high pH alone.

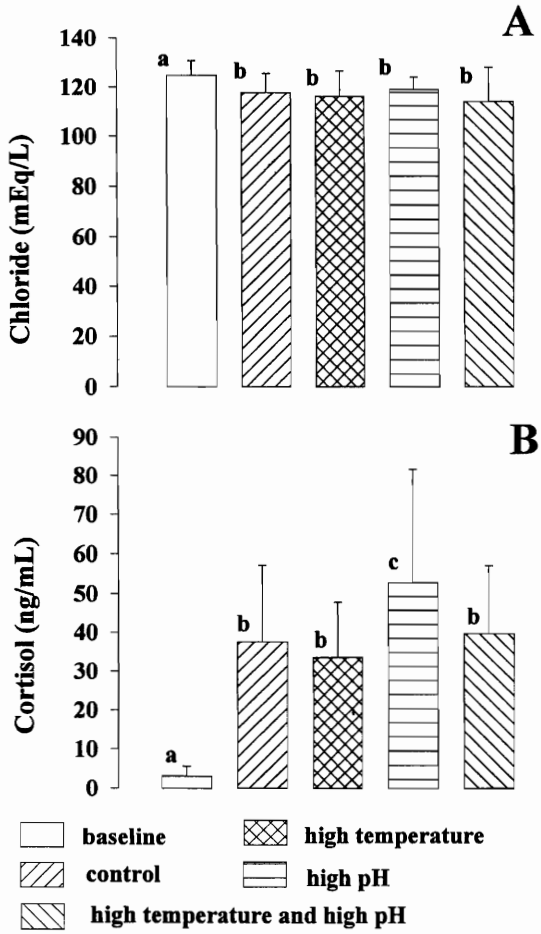

FIGURE 2.—Mean (+SD) **(A)** plasma chloride and **(B)** plasma cortisol concentrations in rainbow trout ($N = 20$) sampled before transport (baseline) or transported and sampled 1.5 h after stocking into raceways of high pH (9.0), high temperature (22°C), high pH and high temperature, or control pH (7.7) and temperature (16°C). Treatments sharing a common letter are not significantly different (Fisher's least-significant-difference test, $P \leq 0.05$).

Laboratory Tests

Experiment 1. High temperature, high pH.—At 1.5 h after stocking, all groups showed significant increases in plasma cortisol (Figure 3) and glucose (Figure 4) compared with baseline levels. Cortisol peaked in the 3-h sample and returned to nearly baseline levels within 6 h in both the high-temperature controls and treatments (Figure 3). Plasma cortisol concentrations were significantly higher than control levels in the high-pH and high-pH–high-temperature treatments. Plasma cortisol, glucose, and chloride (Figure 5) levels in fish from the high-temperature treatment did not differ from levels in the control treatment, except for the 1.5-h and 24-h samples, cortisol concentrations were lower and then higher than control treatment levels.

The high pH and high temperature combination resulted in the highest glucose levels, which were significantly higher than fish from control tanks at 1.5 h (Figure 4). At this time, plasma chloride (Figure 5) was significantly depressed in all

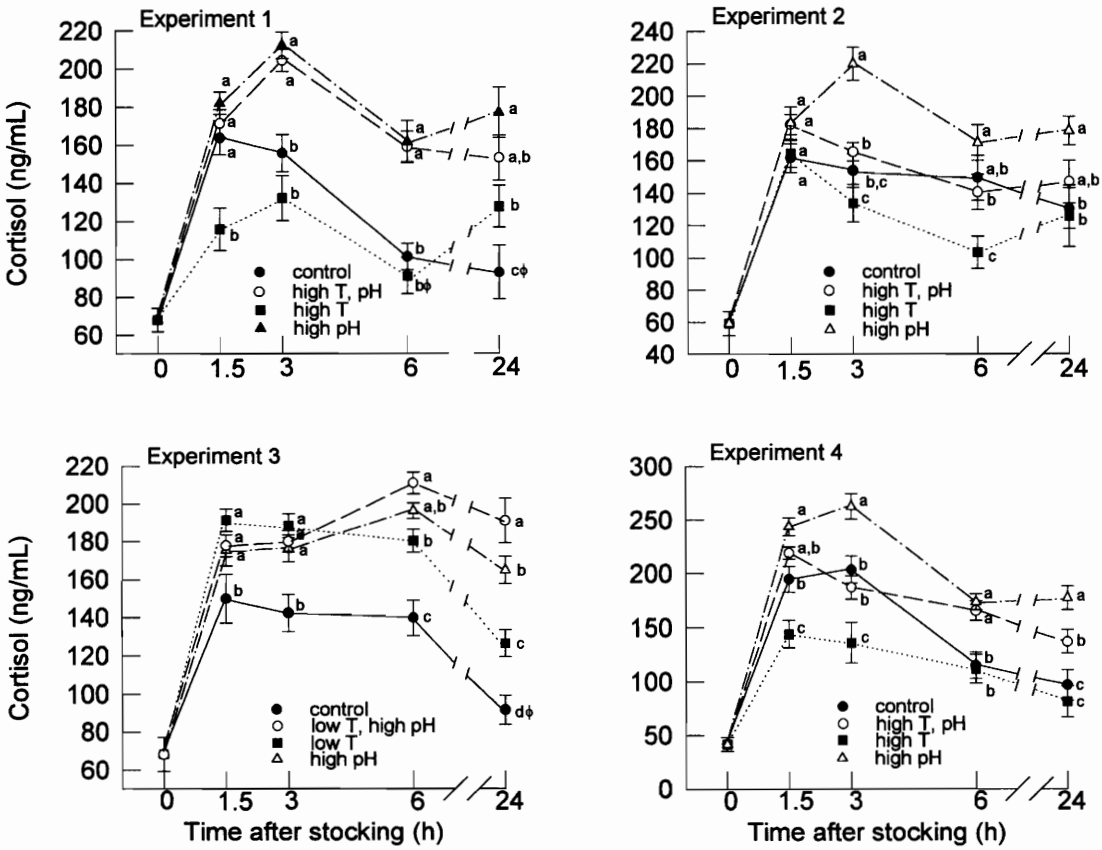

FIGURE 3.—Mean (±SE) plasma cortisol concentrations in rainbow trout sampled before transport (time 0 = baseline) or transported and sampled 1.5, 3, 6, and 24 h after stocking into circular tanks with the various combinations of pH and temperature (T) for the four wet laboratory tests (experiments 1–4, see Methods). Within an experiment at the same sampling time, means sharing a common letter are not significantly different; ϕ indicates a mean that is not significantly different from the baseline ($P \leq 0.05$).

stocked fish compared with the baseline measurements; the lowest concentrations were in the combination group. Additional elevations in plasma glucose (Figure 4) occurred in fish from the high-pH group, in which glucose remained high at 24 h. The response to pH was different at high temperature, with both glucose and chloride concentrations returning to control levels within 24 h.

Plasma chloride levels were significantly depressed in both treatments with high pH, but levels differed with temperature across time (Figure 5). At low temperatures, the high-pH-exposed fish had depressed chloride levels at 3 h and levels remained low at 24 h.

Mortality was significantly higher with the high-temperature and high-pH treatment (100%) than for fish exposed to high pH only (72%). High temperature alone caused no mortality after 96 h, and all fish in the control treatment survived as well (Table 2).

Experiment 2. High temperature, high pH.—At 1.5 h after stocking, all groups showed significant increases in plasma cortisol (Figure 3), and glucose (Figure 4) and decreases in chloride (Figure 5) compared with baseline levels. In the control and high-temperature treatments, plasma glucose and chloride returned to baseline levels by 24 h. However, cortisol levels remained significantly greater than baseline levels in all treatments, even after 24 h (Figure 3).

Cortisol concentrations were significantly greater in the high-pH treatment than in the other treatments at 3 and 24 h (Figure 3). As in Experiment 1, the elevated temperatures of the combination and high-temperature treatments lowered the cortisol response. Cortisol concentrations in the com-

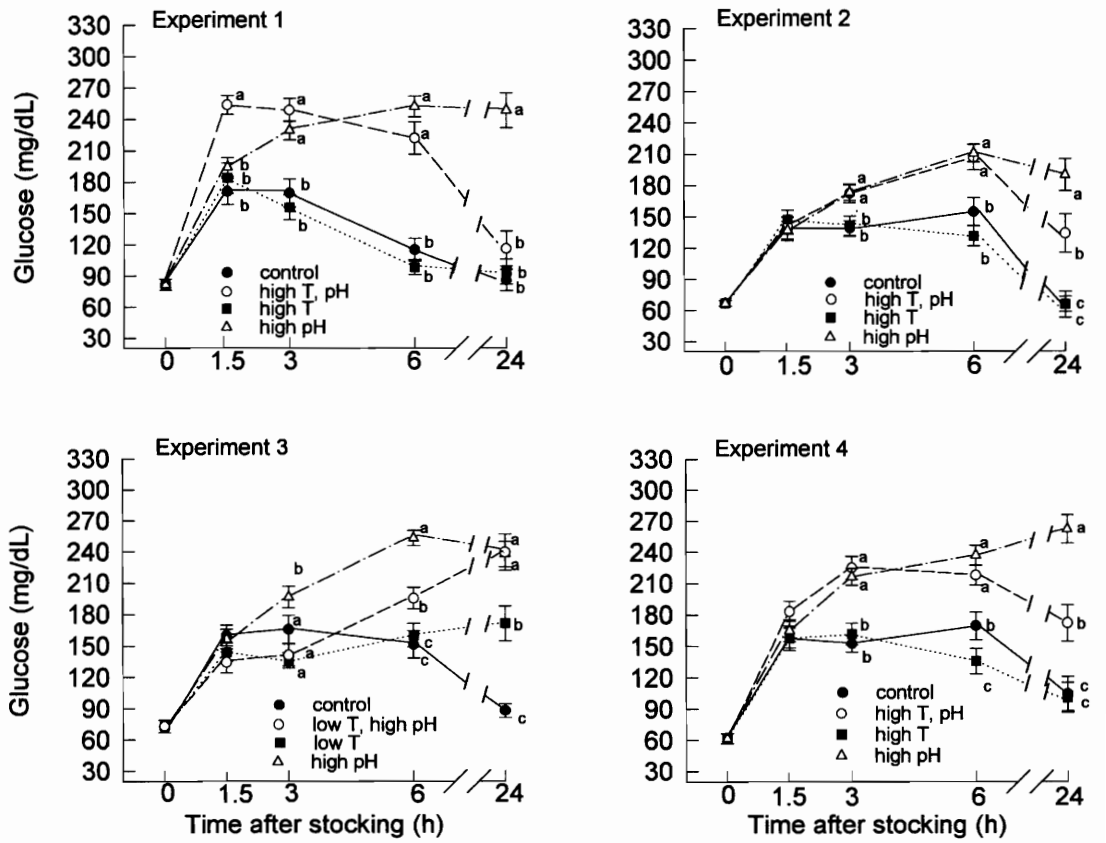

FIGURE 4.—Mean (±SE) plasma glucose concentrations in rainbow trout sampled before transport (time 0 = baseline) or transported and sampled 1.5, 3, 6, and 24 h after stocking into circular tanks with the various combinations of pH and temperature (T) for the four wet laboratory tests (experiments 1–4, see Methods). Within an experiment at the same sampling time means sharing a common letter are not significantly different ($P \leq 0.05$).

bination treatment did not significantly differ from the control treatment. However, glucose concentrations in fish from both the combination and the high-pH treatments were significantly higher than the control and the high-temperature treatments at 3, 6, and 24 h (Figure 4). Plasma chloride differed significantly among the treatments only at 24 h when the high-pH and combination treatments were significantly lower than the control treatment (Figure 5).

Mortality ranged from 5.0% (combination treatment) to 0% (high temperature) and did not differ significantly among treatments (Table 2).

Experiment 3. Low temperature, high pH.—At 1.5 and 3 h after stocking, all groups showed significant increases in plasma cortisol (Figure 3) and glucose (Figure 4) and decreases in plasma chloride (Figure 5) compared with baseline values. Plasma chloride was significantly lower than controls in the combination treatment at 6 and 24 h. Fish exposed to high pH at control temperatures had chloride concentrations at 6 h that were closer to control values than fish exposed to high pH at colder temperatures (Figure 5). However at 24 h, fish in the high-pH treatment still had significantly lower chloride concentrations than control fish, as did fish in the other two treatments.

Plasma cortisol was significantly higher than controls in all treatments at 1.5, 3, 6, and 24 h (Figure 3). At 24 h after stocking, cortisol concentrations in control fish had returned to baseline levels, but levels from the other treatments remained high, albeit reduced from the 6-h sample. Cortisol concentrations were highest in the two treatments with elevated pH and were significantly affected by low temperature (7–9°C); the low temperature in the combination treatment resulted in a higher cortisol level than in the high-pH treatment. Low temperature also caused a stress response independent of pH, as indicated by signif-

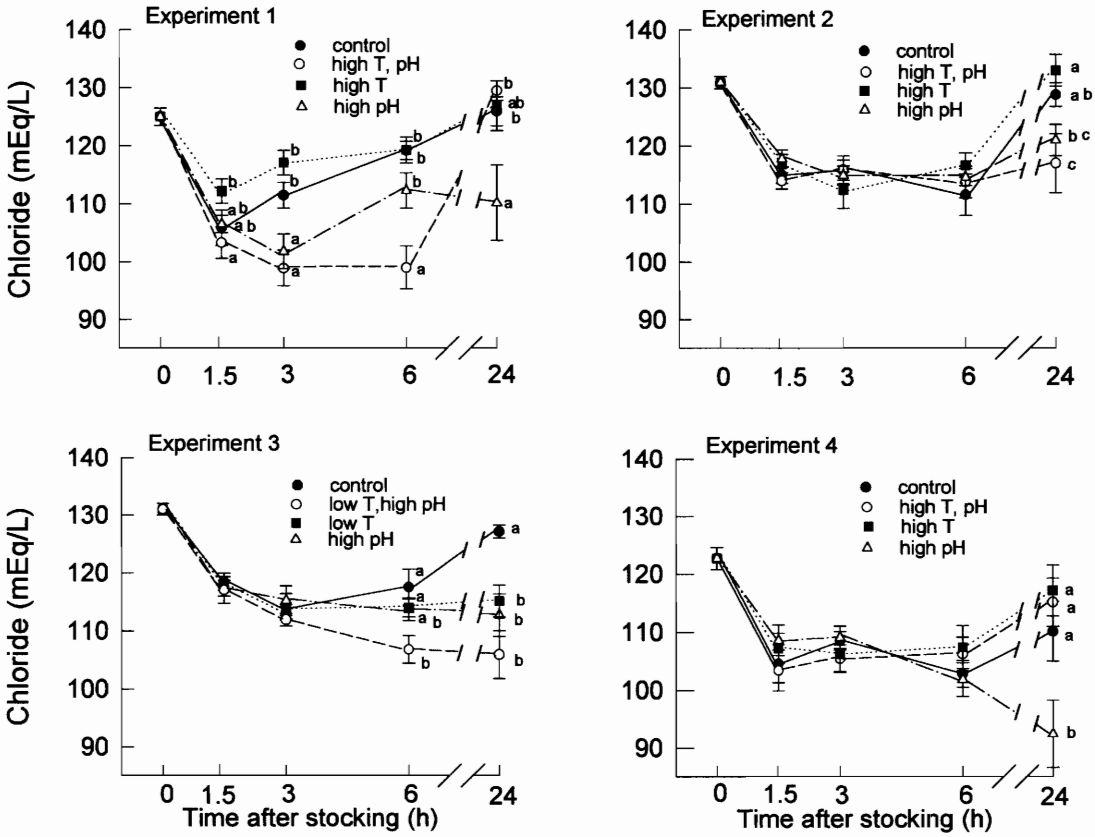

FIGURE 5.—Mean (±SE) plasma chloride concentrations in rainbow trout sampled before transport (time 0 = baseline) or transported and sampled 1.5, 3, 6, and 24 h after stocking into circular tanks with the various combinations of pH and temperature (T) for the four wet laboratory tests (experiments 1–4, see Methods). Within an experiment and at the same sampling time means sharing a common letter are not significantly different (*t*-test, $P \leq 0.05$).

icantly higher cortisol levels in the low-temperature group than in the control group at 24 h. Similarly, plasma glucose at 24 h was highest in both the high-pH and combination treatments, and the low-temperature-treated fish had significantly higher levels than control fish (Figure 4). Mortality (Table 2) was significantly greater in the combination (24.3%) and high-pH treatments (18.6%) than in the control (0%) or low-temperature treatments (0%).

Experiment 4. High temperature, high pH (Ten Sleep strain).—Plasma cortisol increased significantly above baseline levels in all treatments, peaked at 1.5–3 h after stocking, and decreased at 24 h to levels that were still above baseline (Figure 3). Cortisol in fish from the high-pH treatment was significantly higher than in control fish for each time period. The combination treatment also was significantly higher than controls at 6 and 24 h. Fish from the high-temperature treatment had significantly lower cortisol concentrations than controls at 1.5 and 3 h. Cortisol concentrations in the Ten Sleep strain of rainbow trout (this test) tended to be higher than in the Sand Creek strain (used in the other experiments).

Fish from the high-pH and combination treatments had significantly higher glucose concentrations than in control fish at 3, 6, and 24 h (Figure 4). At 24 h, the glucose continued to rise in the high-pH treatment and was significantly higher than in the combination treatment. Plasma chloride levels did not differ from controls at any time except at 24 h in the high-pH treatment (Figure 5).

As in Experiment 1, fish exposed to the combination of high pH and high temperature had significantly greater mortality (81.1%) than fish ex-

TABLE 2.—Percent mortality ($N = 40$ fish for each treatment within each experiment) after 96 h for rainbow trout subjected to different combinations of pH and temperature (temp) in four wet laboratory experiments. Control values for pH (7.8) and temp (14°C) were the same for all experiments, but ranges of high pH and high or low temp varied for each experiment. Experiment 1: high pH, 9.0–9.5; high temp, 19–22°C. Experiment 2: high pH, 8.4–9.6; high temp, 18–20°C. Experiment 3: high pH, 8.6–9.6; low temp, 7–9°C. Experiment 4: high pH, 8.4–9.5; high temp, 20–22°C. Experiments 1–3 were conducted with Sand Creek strain rainbow trout; experiment 4 used Ten Sleep strain rainbow trout. Within an experiment, mean treatment mortalities without a letter in common are significantly different (chi-square analysis, $P < 0.05$).

Treatment	Mean mortality (%) for experiment: 1	2	3	4
Combined high pH with high or low temp	100.0 z	5.0 z	24.3 z	81.1 z
High pH, control temp	71.5 y	3.3 z	18.6 z	36.6 y
High or low temp, control pH	0.0 x	0.0 z	0.0 y	5.6 x
Control temp and pH	0.0 x	1.6 z	0.0 y	0.0 x

posed to high pH alone (36.6%) (Table 2). High-temperature-treatment mortality (5.6%) did not differ significantly from controls (0%).

Field Tests

Survival after 96 h of the rainbow trout stocked into cages is presented in Table 1. Survival in Spirit Lake and Payson Reservoir indicated that the handling protocol and high truck-loading densities did not cause mortality of fish. Mortality of fish in the field generally corroborated the lethal pH and temperature combinations tested in the laboratory. Mortality in the field occurred at pH greater than 9.3–9.4 with temperatures of 19.9–22.8°C; this is based on the minimum pH experienced at the maximum oxygenated depth available to the fish. If fish came from a hatchery with warmer water (e.g., 17.1°C), survival was much better under these conditions.

The pH generally decreased at the bottom of the water column, but it was fairly uniform in the epilimnion, especially under windy conditions. Diel changes in pH at the surface ranged 0.1–0.3 pH units at Spirit Lake, 0.1–0.2 units at Payson Reservoir and Nine Mile Reservoir, and 0.1–0.5 units at Mantua Reservoir.

Cortisol concentrations of fish sampled from cages in Mantua Reservoir were significantly elevated from control levels at 3, 6, and 24 h (Figure 6). Glucose concentrations were also significantly

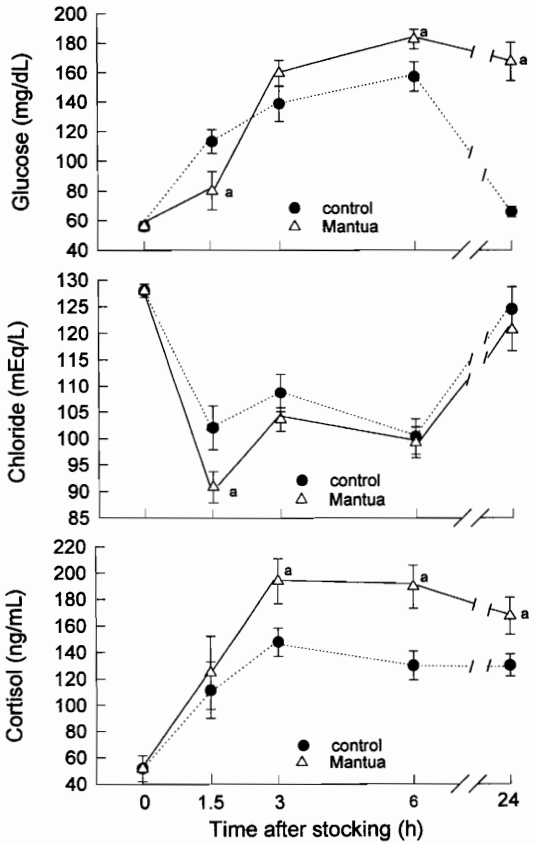

FIGURE 6.—Mean (±SE) plasma chloride, glucose, and cortisol concentrations in rainbow trout sampled before transport (time 0 = baseline) or transported and stocked into cages at Mantua Reservoir or at the hatchery (control) and sampled at 1.5, 3, 6, and 24 h. The letter "a" indicates a mean that is significantly different from the control within that sampling time (t test, $P \leq 0.05$).

higher at 6 and 24 h at the reservoir (Figure 6). At 1.5 h, glucose concentrations were significantly lower in the fish from the reservoir than in control fish. Chloride concentrations were significantly lower in the reservoir fish than in control fish only in the 1.5-h sample. Mortality of caged fish within the reservoir was significantly higher (39%) than for the control fish at the hatchery (3.5%).

Discussion

Other than research by Wilkie et al. (1993) and Wright et al. (1993) evaluating the physiological response of Lahontan cutthroat trout *Oncorhynchus clarki henshawi* to pH 10, the stress response to high pH has received little attention. However, several investigators demonstrated harmful and le-

thal effects on rainbow trout exposed to high pH (Jordan and Lloyd 1964; Witschi and Ziebell 1979; Murray and Ziebell 1984). The threshold pH for mortality appears to vary depending upon acclimation pH, species, and the study. Witschi and Ziebell (1979) observed only one death among 25 rainbow trout after 24 h exposure to pH 9.0. When the pH was increased to 9.3, swimming and appetite decreased. At 9.5, 32% of fish acclimated to pH 7.2 died within 48 h. Jordan and Lloyd (1964) observed 50% mortality in 360-h tests at pH 9.5, but Daye and Garside (1975) observed no mortality in brook trout *Salvelinus fontinalis* at the same pH in 167-h tests.

Acclimation to pH 8.0 for 6 h resulted in 40–50% mortality at pH 9.5 (Murray and Ziebell 1984). Longer acclimation (24 h) at pH 8.0 resulted in 100% survival of rainbow trout at pH levels as high as 9.7–9.9 in 96-h tests (Murray and Ziebell 1984). Wilkie et al. (1993) noted that Lahontan cutthroat trout lived at pH 9.4 in Pyramid Lake, Nevada, but more than half of the fish challenged at pH 10.0 died after 72 h exposure. That some fish can acclimate to high pH is evident in the survival of trout at pH 10.2 in an Arizona lake despite increased blindness and frayed fins (Eischer 1946). Sanborn (1945, cited by Daye and Garside 1975) similarly observed largemouth bass *Micropterus salmoides* and bluegills *Lepomis macrochirus* surviving pH 10.5. In the hot springs of Lake Magadi, Kenya, Magadi tilapias *Oreochromis alcalicus grahami* thrive at pH 9.6–10.4 (Narahara et al. 1996).

In this study, the effect of temperature on the lethal pH limits and the physiological stress response were of interest. The combination of high pH and high or low temperature resulted in significantly higher mortality than for fish exposed to high pH at control temperatures. The stress response to high pH was also significant at values of 9.0 (preliminary test) or greater (laboratory and field tests).

Handling and Stocking Stress

In both field and laboratory tests, plasma glucose and cortisol levels in all treatment groups of transported fish were significantly higher than baseline levels in fish. These results and the decrease in plasma chloride indicated a marked stress response due to transport and handling. This was consistent with other investigations on handling stress (Strange et al. 1977; Barton et al. 1980; Davis and Parker 1983) and transport stress (Barton et al. 1980). Previous studies have also shown a decrease in plasma chloride in response to handling stress (Wedemeyer 1972; Davis and Parker 1983) or hauling stress (Carmichael et al. 1983; Carmichael 1984).

Effect of Temperature on Stress Response to pH

High pH caused significant elevations in plasma glucose and cortisol and decreases in chloride. Cortisol increased significantly at pH values as low as 9.0. The combination of high temperature and high pH did not have a cumulative effect on cortisol concentrations. Instead, plasma cortisol and glucose levels of the combination treatment were often significantly lower than levels in the high-pH group, indicating a more rapid clearance of those compounds or more rapid return to homeostasis at higher temperatures. Strange et al. (1977) noted that peaks in stress-induced cortisol in cutthroat trout *O. clarki* waned much faster (40–70 min after handling) for fish maintained at 23°C than at 9°C. This response at higher temperatures was also noted for plasma glucose and chloride levels in goldfish *Carassius auratus* injected with cortisol (Umminger and Gist 1973). Injected fish at 32°C had lower blood glucose and higher chloride levels than fish at 20°C.

Effect of Temperature Alone on Stress Response

A marked temperature increase can cause a cortisol response. Strange et al. (1977) found a temperature increase from 13°C to 26°C caused a significant increase in cortisol levels of cutthroat trout. Thomas et al. (1986) found temperature cycles of 9–15°C did not induce cortisol increases in juvenile coho salmon *Oncorhynchus kisutch,* whereas 6.5–20°C cycles did. In this study, the temperature increase of 5.3°C was probably not high enough to induce a net cortisol elevation above that induced by transport stress, but it was able to ameliorate some of the cortisol increase associated with the high-pH treatment. The higher temperatures of 19°C and 22°C did not induce additional changes in glucose and chloride levels.

However, there was a significant stress response to the lower temperature. Barton and Peter (1982) noted a similar response by rainbow trout chilled to 1°C. Low temperature (7–9°C) in this study produced significantly higher glucose and cortisol and lower chloride levels when compared with controls at 24 h. In combination with high pH, this temperature did not induce additional elevations in glucose or decreases in chloride concentrations when compared to high pH alone; however, it did

have a noticeable effect in delaying the glucose response.

Strain Differences

Overall, nearly the same results with glucose and chloride occurred between strains despite the size difference. The pattern of mortality was also similar among strains. Although no statistical comparisons between strains were possible, the results suggest no benefits to fisheries management by improving stocking survival by exclusively using either of the strains. However, other strains not tested may be resistant to high pH and may be useful for stocking in Utah waters. Similarly, pH-resistant fish could be recruited from high-pH reservoirs for broodstock. The progeny would potentially have greater survival in eutrophic reservoirs. Lahontan cutthroat trout, adapted to pH 9.4 in Pyramid Lake (Wilkie et al. 1993), may also be an appropriate alternative to rainbow trout for high-pH waters.

Conclusions and Recommendations

High pH values of 9.0 or greater induced a significant stress response. Results did not show an additive or synergistic effect of temperature and pH on the stress response of rainbow trout to hauling and stocking. However, the combination did have an independent, additive effect on mortality. Results indicated that higher temperatures can speed the return of stress indicators to homeostasis, probably by increasing the clearance rate of metabolites. Alternately, cold temperature delayed the stress response and induced a stress response itself. Further tests evaluating the effects of size, species, and acclimation temperatures are recommended.

The water temperatures used in this study were not high enough to induce mortality, except when combined with high pH. Results indicated that survival of stocked fish may be compromised if pH levels greater than 9.3–9.4 are associated with temperatures greater than 19–22°C. The results also stress the need for accurate pH determinations of receiving waters to avoid mortality.

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