

**Causes of Recruitment Bottlenecks in Translocated Cutthroat Trout
Populations: Investigation of Low Temperature Effects**

Mark A. Coleman and Kurt D. Fausch

**Department of Fishery and Wildlife Biology
Colorado State University
Fort Collins, Colorado 80523**



Final Report

March 2006

**Causes of Recruitment Bottlenecks in Translocated Cutthroat Trout
Populations: Investigation of Low Temperature Effects**

Mark A. Coleman
Graduate Research Assistant

and

Kurt D. Fausch
Principal Investigator

Department of Fishery and Wildlife Biology
Colorado State University
Fort Collins, Colorado 80523

Final Report to:

Colorado Division of Wildlife
Fort Collins, Colorado
Interagency Agreement IA-966-2002

Central Utah Project Completion Act Office
U. S. Department of Interior
Provo, Utah
Cooperative Agreement 02-FC-CU-CS030

USDA Forest Service
Fish Habitat Relationships Program
Rocky Mountain Region, Denver, Colorado
Challenge Cost Share Agreement 03-CS-110206000-013

National Park Service
Cooperative Ecosystem Studies Unit
Water Resources Division
Fort Collins, Colorado
Cooperative Agreement H1200-04-0002

March 2006

PROJECT ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the Colorado Division of Wildlife (CDOW) administered by Tom Nesler; the Central Utah Project Completion Act Office, U.S. Department of Interior, administered by Ralph Swanson; the USDA Forest Service, Rocky Mountain Region Fish Habitat Relationships Program, administered by Greg Eaglin and Dave Winters; and the National Park Service, Water Resources Division, administered by Jim Tilmant. Additional support for Mark Coleman came from the John C. and Marietta Peters Scholarship Fund, the Gregory L. (Greg) Bonham Memorial Scholarship Fund, and the West Denver Trout Unlimited Scholarship. Rick Kolecki (CDOW, Glenwood Springs) and Phil Schler (CDOW, Bellvue) provided trout eggs and advice on culture of native cutthroat trout. Steve Brinkman (CDOW, Fort Collins) provided advice and equipment. Pete Walker and Mike Minniear (CDOW, Brush), provided diagnostic services, disease treatment, and fish culture advice. Phil Chapman (Statistics, CSU), and Gary White and Ken Burnham (Fishery and Wildlife Biology, CSU) provided statistical consulting. Brett Johnson and Chris Myrick (Fishery and Wildlife Biology, CSU) provided advice on fish culture and study design. Chris Myrick also generously provided laboratory space at the Foothills Fishery Facility of Colorado State University.

We thank Terry Terrell (National Park Service, Estes Park) for assistance with permits and entry into Rocky Mountain National Park. Doug Laraby and Jack Mason (Winter Park Ski Resort, Winter Park) provided access to Little Vasquez Creek. Information on field sites was provided by Bruce Rosenlund and Chris Kennedy (U.S. Fish and Wildlife Service, Lakewood), Paula Geunther-Gloss, Ashley Ficke, Kris Sexton, Doreen Summerlin, and Mike Young (USDA Forest Service, Fort Collins and Granby), Amy Harig (Trout Unlimited, Boulder), and Greg Horstman. Mike Young (USDA Forest Service, Missoula, MT) also provided feedback on our fry surveys. Jeff Falke provided assistance working with digital elevation models. Field and/or laboratory assistance was provided by: Erica Barr, Colden Baxter, Mandi Brandt, Kendra Brewer, Sarah Cain, Cecelia Coleman, Joe Connery, Mike Del Tonto, Jackie Dieterle, Ashley Ficke, Matt Hopken, Sarah Hughes, Tim Jenson, Angela Klein, Matt Kondratieff, Cinnamon Levi, Leslie Meier, Devin Norcross, Doug Peterson, Kendall Ross, David Rouse, Thalia Sachtleben, Carl Saunders, Dan Scott, Luke Senig, Zach Shattuck, Cassie Tripp, Jeff Yelton, and Lucas Young.

TABLE OF CONTENTS

Executive Summary	1
Introduction.....	7
Project Background	7
Project objectives.....	9
References	9
Part 1: Laboratory Experiment – 2004 Replicate.....	11
Introduction	11
Methods	11
Results	17
Discussion	19
References	21
Tables and figures.....	22
Part 2: Cold Summer Temperature Regimes Cause a Recruitment Bottleneck in Age-0 Colorado River Cutthroat Trout Reared in Laboratory Streams	28
Abstract	28
Introduction	29
Methods	31
Results	39
Discussion	42
Acknowledgments.....	46
References	47
Tables and Figures.....	49
Part 3: Cold Summer Temperature Limits Recruitment of Age-0 Cutthroat Trout in High-Elevation Colorado Streams	60
Abstract	60
Introduction	61
Study sites.....	63
Methods	64
Results	74
Discussion	79
Acknowledgments.....	83
References	84
Tables and Figures.....	88
Appendix A. Histopathology Study.....	102
Appendix B. Table of Fry Counts from Field Study.....	114
Appendix C. Study Stream Mean Daily Temperatures	115

EXECUTIVE SUMMARY

Colorado River cutthroat trout (CRCT; *Oncorhynchus clarkii pleuriticus*) have declined throughout their native range in the headwaters of the Colorado River basin, due to overfishing, habitat degradation, and invasions by nonnative salmonids (Behnke 1992; Young et al. 1996). Estimates of their current distribution have varied, but the most recent estimates suggest that CRCT now occupy about 14% of their historic native range (Hirsch et al. in press). Due to their decline, CRCT were considered a Federal Category 2 “candidate species” proposed for listing under the Endangered Species Act (ESA) before the categories were dropped in 1996 (CRCT Conservation Team 2006). Although no longer federally listed under the ESA, CRCT have been granted special status by the states of Colorado, Utah, and Wyoming, and the U.S. Forest Service and Bureau of Land Management. These state and federal agencies formed the CRCT Conservation Team (CRCT Conservation Team 2006) to coordinate efforts to increase the distribution and abundance of CRCT, and thereby reduce or eliminate the threats that warrant its special status.

One strategy used by fisheries managers to prevent listing has been to establish new populations by translocating genetically pure CRCT to streams where no trout occurred originally (e.g., above natural barriers), or where nonnative trout (usually brook trout, *Salvelinus fontinalis*) that displaced CRCT were removed. One objective of the CRCT Conservation Agreement (CRCT Conservation Team 2006) is to increase the number of stream populations of CRCT in the three states, by restoring CRCT to streams within their native range. Managers report that current restoration projects cost \$50,000 to \$100,000 or more, so these translocations will require substantial resources to complete. The primary goal of this research was to identify

ways to increase translocation success of native cutthroat trout, and thereby reduce the cost of time and money that often hinders such conservation efforts.

With regard to habitat, Harig and Fausch (2002) reported that low summer stream temperature was correlated with low translocation success of native cutthroat trout in 27 streams in Colorado and New Mexico. These streams made up nearly the entire set of 28 streams where translocations of greenback (*O. c. stomias*) and Rio Grande cutthroat trout (*O. c. virginalis*) had been attempted up to 1995. Harig and Fausch (2002) hypothesized that cold summer temperatures delay egg development, fry emergence, and growth during the first summer of life, thereby reducing overwinter fry survival in the harsh, cold environments of high-elevation streams where most translocations have been made. However, this hypothesis has never been tested in the lab or field.

In 2002, we began a focused investigation of the role of low summer stream temperatures as a factor limiting age-0 cutthroat trout fry recruitment through the first summer and the beginning of winter. Using an integrated laboratory and field approach, we studied the effects of low summer stream temperatures on age-0 cutthroat trout fry growth and recruitment to the end of their first growing season. The main laboratory experiment was conducted during 2003, and was replicated during 2004 to include histopathology analyses by a third party (Charlie E. Smith, Fish Pathologist, Bozeman, Montana, U.S. Fish and Wildlife Service, Retired) on fry sampled from experimental tanks during the experiment. The concurrent field study was conducted from 2002-2004.

The laboratory experiments investigated whether cold summer temperatures can limit translocation success in Colorado River cutthroat trout *O. c. pleuriticus* by hindering recruitment of age-0 fry from hatching through the start of winter. We subjected newly hatched fry to one of

three temperature regimes (cold, intermediate, or warm), which averaged 7°C, 8.5°C and 10°C, respectively, during the warmest summer month. The regimes mimicked those of natural streams where translocated fish had died out, or produced populations of cutthroat trout of low or high abundance. In the 2003 experiment, mean fry survival was high during hatching to swimup, ranging from 97% in the warm regime to 85% in the cold, and was significantly lower in the cold

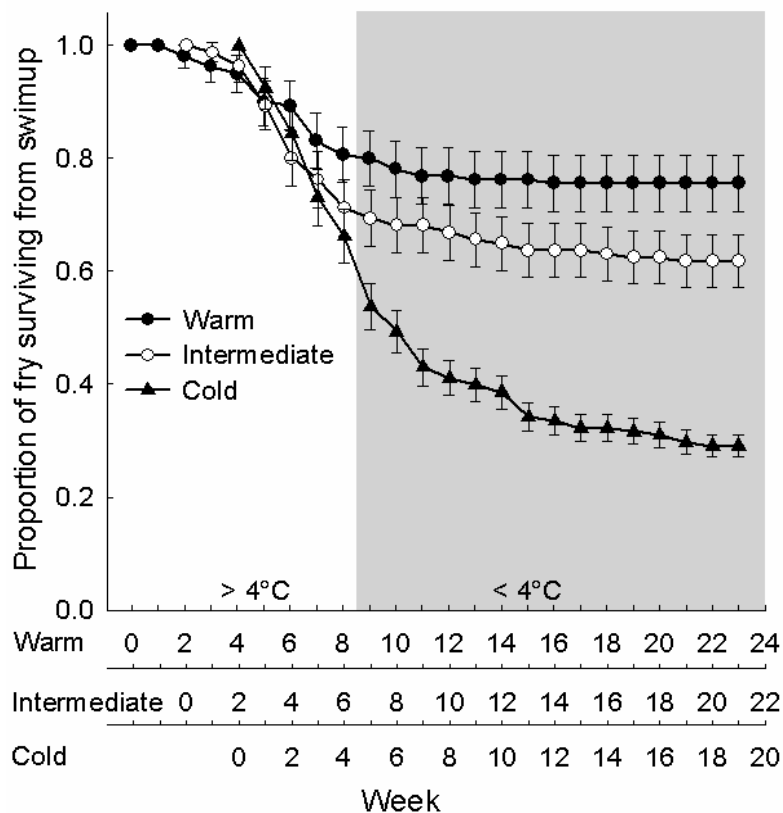


Figure 1. Proportion of age-0 Colorado River cutthroat trout surviving from swim-up to each week during the 2003 laboratory experiment. Bars show Kaplan-Meier 95% confidence intervals.

winter temperatures (Figure 1). The 2004 replicate corroborated this severe recruitment bottleneck. The histopathology study conducted on fry from the 2004 experiment suggested that low energy reserves contributed to many mortalities, and were prevalent in moribund and randomly sampled fry from the cold temperature regime. Thus, while many mortalities in all

regime than either the intermediate or warm regimes. After swimup, fry in the warm regime grew more than 60% larger on average than those in the cold regime by the onset of winter. Survival rates were also higher in the warm regime (76%) than in the intermediate (62%) and cold (29%) regimes through mid-winter. Most mortality in all treatments occurred during a recruitment bottleneck during the 4-week period before the onset of

treatments were related to low energy stores, there was also evidence that fish stored less energy in the cold temperature regime.

During 2002 to 2004 we conducted a field study to measure abundance and size of age-0

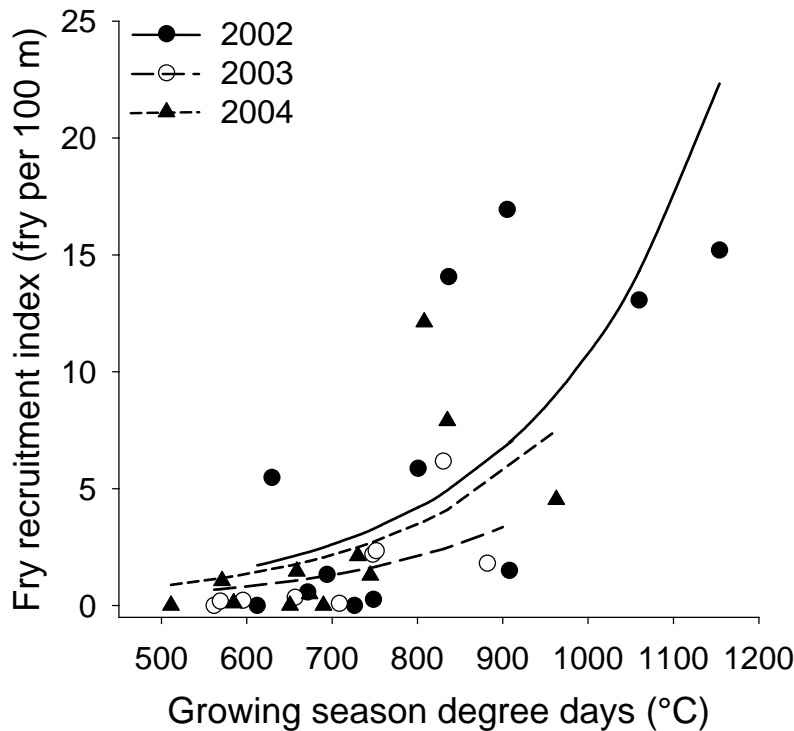


Figure 2. Predicted recruitment of age-0 cutthroat trout fry to the start of winter (fry recruitment index) as a function of growing season degree days for the three study years. Fry recruitment index values are the estimated number of fry surviving to onset of winter based on the number of newly-emerged fry counted.

cutthroat trout fry (greenback *O. c. stomias* or Colorado River cutthroat trout *O. c. pleuriticus*) in six headwater mountain streams in north central Colorado that varied in thermal characteristics. Fry were measured at peak emergence during three years in two 500-m reaches widely spaced in each stream. Fewer fry were observed in the three cold streams, where predicted abundance was low, than in the three warmer streams

where predicted abundance was high. Abundance and body size at peak emergence were adjusted using laboratory data to estimate the number and length of age-0 cutthroat trout surviving to the onset of winter in the six streams, because the length of growing season remaining at the time of peak emergence varied with temperature among streams (i.e., more growing season remained in warmer streams). These estimates of recruitment and the size of fry at the onset of winter increased with Celsius degree days accumulated during the growing

season, and varied among years (Figures 2 and 3). A further exploratory analysis showed that spring snowpack and summer precipitation accounted for additional annual variation beyond that

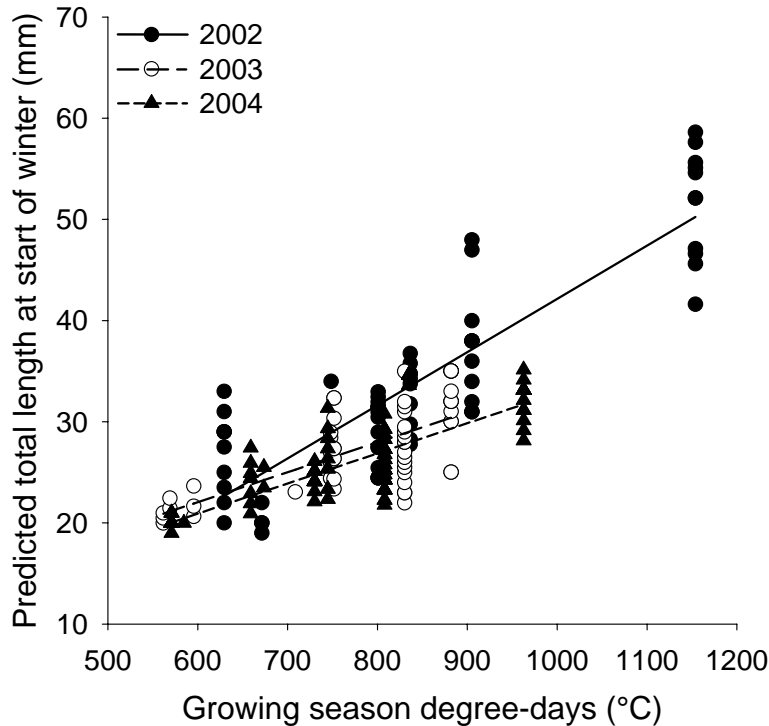


Figure 3. Total length of age-0 cutthroat trout fry at the onset of winter (predicted from lab and field data) as a function of growing season degree days for the three study years. Each point represents the predicted total length at the onset of winter of fish measured during our fry surveys, and the lines plotted are the linear best fits.

explained by temperature.

Our results support the hypothesis that recruitment of these subspecies of native cutthroat trout in Colorado can be limited by cold water temperatures that reduce growth and survival. Cold summer temperatures may cause a severe recruitment bottleneck during the critical period after swim-up in age-0 cutthroat trout that hampers the success of translocations. In addition, our laboratory experiments, combined

with results from previous research (Peterson and Fausch 2002), indicated that age-0 fry that fail to reach 30-35 mm by the onset of winter are unlikely to survive over winter to age 1, so cold temperature limitation of growth may itself reduce recruitment. Generally, high-elevation streams like those we studied that accumulate 900-1200 Celsius degree days during the growing season afford the best opportunity for cutthroat trout fry recruitment and translocation success. Streams that provide 700-900 degree days likely sustain recruitment in only some years, and those with less than 700 degree days are unsuitable for translocations due to regular recruitment

failures.

- Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6.
- CRCT Conservation Team. 2006. Conservation agreement for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) in the States of Colorado, Utah, and Wyoming. Colorado Division of Wildlife, Fort Collins. 15 p.
- Harig, A. L. and K. D. Fausch 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12: 535-551.
- Hirsch, C. L., T. P. Nesler, and S. Q. Albeke. In press. Range-wide status of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). Colorado River Cutthroat Trout Conservation Team Report. Craig, Colorado.
- Peterson, D. P., and K. D. Fausch. 2002. Demography, dispersal, and effects of nonnative brook trout on native cutthroat trout in Colorado streams. Final report to Colorado Division of Wildlife, Central Utah Project Completion Act Office, U.S.D.A. Forest Service, and Trout Unlimited, Fort Collins, Colorado.
- Young, M. K., R. N. Schmal, T. W. Kohley, and V. G. Leonard. 1996. Conservation status of Colorado River cutthroat trout. U. S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-282, Fort Collins, CO.

INTRODUCTION

Project Background

Colorado River cutthroat trout (CRCT; *Oncorhynchus clarkii pleuriticus*) have declined throughout their native range in the headwaters of the Colorado River basin, due to overfishing, habitat degradation, and invasions by nonnative salmonids (Behnke 1992; Young et al. 1996). Estimates of their current distribution have varied, but the most recent estimates suggest that CRCT now occupy about 14% of their historic native range (Hirsch et al. in press). Due to their decline, CRCT were considered a Federal Category 2 “candidate species” proposed for listing under the Endangered Species Act (ESA) before the categories were dropped in 1996 (CRCT Conservation Team 2006). Although no longer federally listed under the ESA, CRCT have been granted special status by the states of Colorado, Utah, and Wyoming, and the U.S. Forest Service and Bureau of Land Management. These state and federal agencies formed the CRCT Conservation Team (CRCT Conservation Team 2006) to coordinate efforts to increase the distribution and abundance of CRCT, and thereby reduce or eliminate the threats that warrant its special status.

One strategy used by fisheries managers to prevent listing has been to establish new populations by translocating genetically pure CRCT to streams where no trout occurred originally (e.g., above natural barriers), or where nonnative trout (usually brook trout, *Salvelinus fontinalis*) that displaced CRCT were removed. One objective of the CRCT Conservation Agreement (CRCT Conservation Team 2006) is to increase the number of stream populations of CRCT in the three states, by restoring CRCT to streams within their native range. Managers report that current restoration projects cost \$50,000 to \$100,000 or more, so these translocations

will require substantial resources to complete. The primary goal of this research was to identify ways to increase translocation success of native cutthroat trout, and thereby reduce the cost of time and money that often hinders such conservation efforts.

With regard to habitat, Harig and Fausch (2002) reported that low summer stream temperature was correlated with low translocation success of native cutthroat trout in 27 streams in Colorado and New Mexico. These streams made up nearly the entire set of 28 streams where translocations of greenback (*O. c. stomias*) and Rio Grande cutthroat trout (*O. c. virginalis*) had been attempted up to 1995. Harig and Fausch (2002) hypothesized that cold summer temperatures delay egg development, fry emergence, and growth during the first summer of life, thereby reducing overwinter fry survival in the harsh, cold environments of high-elevation streams where most translocations have been made. However, this hypothesis has never been tested in the lab or field.

In this final project report, we summarize the results of a three-year integrated laboratory and field study to investigate the effects of cold summer temperature on growth and recruitment of age-0 cutthroat trout between hatching and the start of winter. Part 1 of this report summarizes the results of the final laboratory experiment we conducted during 2004 to replicate a similar experiment completed during 2003, which is described in detail in Part 2 and Coleman and Fausch (in review a). In both 2003 and 2004, we subjected newly hatched Colorado River cutthroat trout fry to three temperature regimes that covered a range similar to those where translocations of greenback and Rio Grande cutthroat trout had resulted in absent, low-, or high-abundance populations (Harig and Fausch 2002; Coleman and Fausch in review a). Part 3 summarizes our field study, in which we monitored temperatures and conducted surveys to estimate fry abundance and size at the start of winter in six streams where we predicted cutthroat

trout recruitment would be low or high based on summer temperature regimes (Harig and Fausch 2002; Coleman and Fausch in review b).

Project objectives

The objective of the laboratory experiments were to test the effects of low water temperature on cutthroat trout fry growth and survival in the laboratory under controlled conditions, relate it to the findings in the field, and to investigate the mechanisms underlying cold-temperature limitation of recruitment in Colorado River cutthroat trout. The objective of the field study was to relate recruitment (i.e., abundance) of fry of native cutthroat trout to stream temperatures. Together, the two studies serve to test the overall hypothesis of Harig and Fausch (2002) that low translocation success of native cutthroat trout is caused in large part by low summer water temperatures, to evaluate the mechanisms of this recruitment bottleneck, and to provide more refined guidelines for the temperature requirements for successful translocations of native cutthroat trout in the southern Rocky Mountain region.

References

- Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6.
- Coleman, M. A., and K. D. Fausch. in review a. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River cutthroat trout reared in laboratory streams.
- Coleman, M. A., and K. D. Fausch. In review b. Cold summer temperature limits recruitment of age-0 cutthroat trout in high-elevation Colorado streams. Transactions of the American Fisheries Society.
- CRCT Conservation Team. 2006. Conservation agreement for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) in the States of Colorado, Utah, and Wyoming. Colorado Division of Wildlife, Fort Collins. 15 p.
- Harig, A. L. and K. D. Fausch 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12: 535-551.

- Hirsch, C. L., T. P. Nesler, and S. Q. Albeke. In press. Range-wide status of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). Colorado River Cutthroat Trout Conservation Team Report. Craig, Colorado.
- Young, M. K., R. N. Schmal, T. W. Kohley, and V. G. Leonard. 1996. Conservation status of Colorado River cutthroat trout. U. S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-282, Fort Collins, CO.

PART 1: LABORATORY EXPERIMENT – 2004 REPLICATE**Introduction**

During 2004, we replicated the laboratory survival study conducted during 2003 (Part 2; Coleman and Fausch in review a). We again subjected age-0 Colorado River cutthroat trout to three temperature regimes in laboratory streams, and measured their growth and survival from swimup to the start of simulated winter temperatures. Each temperature regime mimicked closely a set of natural streams where Harig and Fausch (2002) found that translocated cutthroat trout populations attained either high abundance, low abundance, or died out. We measured survival in each artificial stream from hatching to >50% swimup, and then weekly after swimup through the winter period. The number of swim-up fry used in the experiment was increased based on 2003 data to allow periodic sampling of fry for analysis of lipids and histology without severely depleting the remaining sample needed for study of survival among treatments.

Methods

We tested the effect of three natural temperature regimes on growth and survival of age-0 Colorado River cutthroat trout from hatching through mid-winter during a 17-week laboratory experiment that consisted of two phases. The first (Phase 1) encompassed the early larval stage from hatching to swimup, when fish are referred to as ‘sac fry’. The second (Phase 2) encompassed the late-larval and early juvenile stages from swimup through mid-winter. Because translocation success was lower in high-elevation streams in Colorado with colder summer temperatures (Harig and Fausch 2002), we again predicted that survival rates would be lower in colder temperature regimes. Further, based on results of our 2003 laboratory experiment (Part 2;

Coleman and Fausch in review a), we predicted that a recruitment bottleneck would occur during Phase 2 of the experiment, and that the severity of the bottleneck would increase in colder temperature regimes.

Subjects and acclimation.- On 2 July 2004, we obtained eyed eggs of Nanita Lake Colorado River cutthroat trout (Colorado Division of Wildlife, Glenwood Springs Hatchery). The eggs were maintained at a constant temperature of 8.0°C for 29 days at the hatchery, then shipped via overnight courier to Colorado State University. Upon receipt, the eggs were acclimated from 8.0°C to 7.0°C over a 30-minute period, sanitized in PVP solution (100 mg/L, Providone iodine in water), and incubated at 7.5°C until hatching. Upon hatching, sacfry were transferred into each of 24 artificial streams to begin Phase 1 of the experiment on survival rates from hatching to swimup. To begin Phase 1, we acclimated sacfry to experimental tanks for 1 hour in plastic bags, due to a 0.5-3°C difference between incubation and starting temperatures of the experimental regimes (see below).

Temperature regimes.- We used the three target temperature regimes synthesized for the 2003 experiment (Figure 2.1) to mimic those of relatively cold high-elevation streams where cutthroat trout translocations had resulted in absent, low, or high abundance populations (Harig and Fausch 2002; Part 2; Coleman and Fausch in review a). The three synthesized temperature regimes mimicked the growing season temperatures of natural streams with mean August temperatures evenly spaced at approximately 7.0°C, 8.5°C, and 10.0°C. Thermographs were placed near the inlet and outlet of two streams in each regime during 2004, to monitor the range of temperature variation that fish were subjected to in the artificial streams during the study (Onset Tidbit dataloggers, Onset Computer, Bourne, Massachusetts). Based on data from these thermographs, mean weekly tank temperatures differed from those planned only slightly

throughout the study, averaging 0.2°C (SE = 0.14) lower than planned in the warm regime, and 0.60 °C (SE = 0.07) and 0.40 (SE = 0.07) higher than planned in the cold, and intermediate regimes, respectively.

Artificial stream apparatus.- We used the same apparatus used in the 2003 experiment for the 2004 replicate (Part 2). This apparatus consisted of 24 artificial streams (8 for each temperature regime) to which water was supplied at a rate of 4.0 L/min. Temperature control was likewise accomplished using the same water chilling system and adjustable thermostatic mixing valves used during the 2003 experiment (Part 2). The tanks were gently cleaned with a siphon weekly to remove excess food and other detritus during the study.

Phase 1 .- To determine survival rates of cutthroat trout fry from hatching to swimup in each temperature regime, we placed 101 sacfry within 5 days of hatching in each artificial stream. Sacfry were held in screen boxes (28 cm by 18 cm by 10 cm deep) with opaque covers to shield them from light, prevent burrowing into the gravel, and allow daily mortality counts. We started the experiment at the point in each synthesized temperature regime that matched the number of degree days to which the eggs and fry had already been exposed since spawning (egg take). We estimated the spawning date as the day in each regime that temperatures first exceeded 5°C, because this is the temperature at which native cutthroat trout have been observed to spawn in Colorado (USFWS 1998; B. Rosenlund, U.S. Fish and Wildlife Service, Lakewood, Colorado, personal communication).

We recorded mortalities daily, and estimated the proportion of fish that had reached swimup. During the first two days, mortalities were assumed to be caused by handling, and were replaced with extra hatchling fry that were maintained at the incubation temperature (7°C) for this purpose. Phase 1 for each treatment ended when we judged that >50% of sacfry had reached

swimup (i.e., the median swimup time). We considered fish to have reached swimup when external signs of remaining yolk were no longer visible, and fish held positions in the water column rather than resting on the bottom. We estimated the proportion of fry reaching swimup after observers remained motionless for 2 minutes, to allow fish to become acclimated to their presence. In addition, we collected a sample of 48 surplus hatchling fry at the start of Phase 1, one sacfry per tank (24 total) at the estimated midpoint of Phase 1, and two swimup fry per tank (48 total) at the end of Phase 1 for planned determination of baseline lipid content during sacfry development.

Phase 2.- At >50% swimup in each treatment, we selected 37 swimup fry randomly from the basket in each tank, and measured their total lengths (TL) and wet weights. We released these fry into the stream tank and euthanized remaining fry in all but one tank in each treatment. These ‘extra’ fry were used to replace mortalities during the first two days of Phase 2, which we assumed were due to acute effects of handling. The number of fry per tank was increased in 2004 to accommodate sampling of fish during Phase 2 for lipid and histopathology analyses. Due to the different rates of development in the different temperature regimes, the start of Phase 2 was synchronized to life-history stage (50% swimup) for each treatment, rather than calendar date. As a result, Phase 2 began 14 days later in the cold treatment, and 7 days later in the intermediate treatment, than in the warm treatment (Figure 2.1).

Tanks were inspected daily for mortalities, which were removed and frozen in water. To measure survival, we carefully counted surviving fish each week. Moribund fish, which had taken on a dark gray or black coloration over the caudal half or over their entire body and had lost equilibrium were counted as mortalities, euthanized, and either frozen in water for planned lipid analyses, or preserved for subsequent histopathology study. We considered any missing

fish mortalities in our weekly counts, because they could not escape from tanks. We measured the total length and wet weight of each surviving fish at the end of the experiment, after 5-6 weeks of simulated winter temperatures. Capture and measurements procedures were identical to that of the 2003 experiment (Part 2). Fish were not measured at the onset of winter during the 2004 replicate because higher mortality rates had occurred in the preceding weeks, and we were concerned that mortality due to handling might reduce diminish fish numbers in the cold treatment and jeopardize our planned analyses. At the end of Phase 2, we euthanized and froze a sample of the surviving fish from each temperature regime for lipid analysis, and preserved an additional sample for histopathology study.

Fish health.- During the original experiment in 2003, a parasite infestation (*Ichthyobodo* spp.) was discovered and successfully treated (Part 2, Coleman and Fausch in review a). The infestation was coincident with the sharp rise in mortality during the recruitment bottleneck, and some mortality due to the parasite itself could not be ruled out. During 2004, moribund fish were examined when mortality rose sharply again during the four to five weeks after swimup, but no parasite infestation was observed.

Feeding.- When the first fry reached swimup near the end of Phase 1, fish were fed Rangen Trout Starter Diet #0 (Rangen Feeds, Boise, ID) ad libitum once or twice daily. During Phase 2, fish were fed 75% of the optimum ration of Rangen Trout Starter Diet daily in two increments by sprinkling food over the inlet flow to simulate invertebrate drift. The optimum ration was calculated each week based on treatment temperature, fish length, and the number of fish remaining using a commercial feeding chart. The feed quantity determination and rationale were otherwise the same as that reported for the 2003 experiment (Part 2, Coleman and Fausch in review a).

Lipid Analysis.- An attempt was made to adapt a procedure using lipid extraction and gas chromatography to determine lipid content of individual fry. Individual fry are much smaller and vary greatly in size compared to the fixed quantities of fish tissues prescribed for standard lipid determination procedures using these techniques. The small and highly variable size of the fry added complexity to the extraction and lipid estimation procedures. These additional complexities created unforeseen difficulties and unfortunately the analysis failed to yield results.

Histopathology Study.- During the 2004 replicate, a random sample of fish was collected from each of the three temperature regimes at swimup, the start of winter, and at the end of the experiment. These and several moribund fish removed from tanks as mortalities throughout the study were euthanized, preserved in Bouin's fixative (Presnell et al. 1997), and stored until January 2005. These samples were then sent to Colorado Histoprep (Fort Collins, Colorado) for histological processing, which included imbedding whole fish in paraffin, microsectioning, and preparation of microscope slides. They were then forwarded to Charlie E. Smith (US Fish and Wildlife Service, Retired, 212 Story Hill Rd, Bozeman, MT 59715) for histological analysis (Appendix A).

Data analysis.- For Phase 1, we used one-way analysis of variance (ANOVA) with posthoc least squares means (LSMEANS) comparisons to test for statistical differences in survival among treatments (PROC GLM, SAS v9.1). Such differences could be due to the life stage duration alone (assuming constant daily mortality rate), differences in daily mortality rate, or both (Houde 1987). Therefore, we analyzed arcsine squareroot transformed survival and daily mortality rates (the proportion that died divided by the duration of Phase 1 in days) through >50% swim-up for each treatment.

For Phase 2, we compared the total lengths and wet weights of fish among treatments

using a linear model with repeated measures, and used the Satterthwaite method to calculate denominator degrees of freedom (PROC MIXED, SAS v9.1). Because fish were not individually identified, the tank was the experimental unit for this analysis, and the size data were log transformed to correct for the variance increasing with size. We compared survival rates among treatments for Phase 2 using the normal approximation of the binomial, by calculating Kaplan-Meier 95% confidence intervals (Newman 1995) on survival from swimup to each week of Phase 2.

Results

Phase 1: Hatching to swimup.- Results from the 2004 laboratory survival study show a significant effect of temperature on survival from hatching to swimup (Table 1.1). Survival was significantly different among regimes (ANOVA: $P = 0.02$), but in contrast to our 2003 results, it was the intermediate regime that had significantly lower survival than both the cold (LSMEANS: $P = 0.008$), and the warm (LSMEANS: $P = 0.0008$) regimes, which were not significantly different. Daily mortality rates also differed significantly among regimes (ANOVA: $P = 0.03$), with the intermediate regime significantly lower than the cold (LSMEANS: $P = 0.001$) and warm (LSMEANS: $P = 0.008$) regimes, which were not significantly different. The time required to >50% swimup varied: 26 days in the warm regime, 32 days in the intermediate regime, and 40 days in the cold regime.

Phase 2: Swimup through end of study.- During Phase 2, the pattern of survival among treatments (Figure 1.1) was similar to that observed in the 2003 experiment (Part 2; Coleman & Fausch in review). These trends in survival continued throughout the simulated summer, fall, and through 5-6 weeks at winter temperatures (<4°C). However, final survival in the 2004

replicate was 20-45% lower than in 2003. Thus, survival was lower in the 2004 replicate, when there was no parasite infestation or unusual disease outbreak, than in 2003, when a parasite infestation occurred, so parasites are unlikely to account for the observed mortalities.

The pattern of growth among treatments during Phase 2 of the 2004 replicate was also similar to that observed in 2003. Swimup fry in all treatments started at about the same size, but both total length and wet weight had diverged by the end of the study, as indicated by a significant period by temperature regime interaction (ANOVA: $P < 0.0001$). Fish in all three temperature regimes had grown significantly in both length and weight (LSMEANS: $P < 0.0001$ for each comparison), and differed significantly in size by the end of the experiment (LSMEANS: $P < 0.0001$ for each comparison), indicating that growth increased with regime temperature. Warm regime fish were 40% longer than cold regime fish by the end of the study (Figure 1.2). Overall growth was lower in 2004 than in 2003 (compare Figure 1.2 to Figure 2.2).

Histopathology Results.- Histopathology results indicated differences among fish in our three temperature regimes that suggested a lack of nutrition contributed to the mortality of many of the moribund fish collected, particularly cold temperature regime. Despite preliminary observations in 2003 that some moribund swim-up fry in our cold treatment appeared to be starving to death while retaining some yolk (Part 2, Coleman and Fausch in review a), no clear pattern was observed among treatments in the amount of yolk present in randomly sampled fish (Figure 1.3). However, a lower proportion of moribund fish from the cold treatment had yolk in the viscera than those in the intermediate and warm treatments, where half of the fish observed contained small amounts of yolk. Visceral fat deposits were uncommon in fry sampled randomly at swimup in all three temperature regimes. Fat was more prevalent at the start of winter in our intermediate and warm temperature regimes, but notably reduced in fish from the

cold regime, and moribund fish from all three regimes, indicating poor energy reserves in these groups. Some mild degenerative changes were observed in kidney hematopoietic tissue that was also more pronounced in the fish from the cold temperature regime, and abnormal muscle tissues were also reported in fish from the cold and intermediate regimes (see Appendix A).

Examinations of gill tissue revealed nothing extraordinary.

Discussion

The results of the 2004 replicate of our laboratory experiment corroborated the 2003 results and showed a severe recruitment bottleneck during the critical period after swimup in age-0 cutthroat trout fry that is more severe in colder temperature regimes. During this critical period, fry must transition from obtaining all or most of their energy from endogenous yolk stores to consuming exogenous prey to meet energetic requirements, a transition marked by high rates of mortality in many fishes (Houde 1987; 2002). As in the 2003 experiment (Part 2, Coleman and Fausch in review a), fish growth was also reduced in colder temperature regimes.

Lower growth and survival rates in the 2004 experiment than in 2003 may have been due to the slightly colder temperature regimes achieved during 2004, and slightly colder incubation temperatures at the Glenwood Springs Hatchery before we obtained the eggs. Hatchery incubation temperatures for eggs obtained during 2003 were reported to average 8.8°C daily (Rich Kolecki, Colorado Division of Wildlife, personal communication), whereas hatchery incubation temperatures reportedly averaged 8.0°C during 2004 (Dave Davis, Colorado Division of Wildlife, personal communication). Variation between years in temperature regimes at the Glenwood Springs Hatchery may also have resulted in differences in egg quality through effects on vitellogenesis in parental fish.

The histopathology analysis confirmed that age-0 cutthroat trout raised in our cold temperature regime did not store as much lipid in the form of visceral adipose deposits relative to those from the intermediate and warm temperature regimes. However, there was little evidence of differences in yolk remaining at swimup, despite observations of remaining yolk in several moribund swimup fry during our 2003 experiment. Notable was the observation that muscle tissues were abnormal in some fish from the cold and intermediate treatment. Thus, there was histological evidence of abnormal growth and development and reduced energy storage at colder temperatures.

Combined, these results lend additional support to the conclusions of previous research in our laboratory (Harig and Fausch 2002; Part 2; Coleman and Fausch in review a; Part 3; Coleman and Fausch in review b). They suggest that both time and temperatures may not be adequate for normal development and sufficient energy storage to insure recruitment in streams with cold summer temperature regimes like those where cutthroat trout translocations have resulted in only absent or low-abundance populations (Harig and Fausch 2002). Such streams have summer temperatures below about 8°C, and our results agree with those of Harig and Fausch (2002), which indicated that these streams should be considered unsuitable for translocations of cutthroat trout.

References

- Coleman, M. A., and K. D. Fausch. in review a. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River cutthroat trout reared in laboratory streams.
- Coleman, M. A., and K. D. Fausch. In review b. Cold summer temperature limits recruitment of age-0 cutthroat trout in high-elevation Colorado streams. *Transactions of the American Fisheries Society*.
- Harig A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12:535-551.
- Houde, E. D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2:17-29.
- Houde, E. D. 2002. Mortality. Pages 64-87 *in* L. A. Fuiman, and R. G. Werner, editors. *Fishery science: the unique contributions of early life stages*. Blackwell Science, Oxford, UK.
- Presnell, J. K., M. P. Schreibman, and G. L. Humason. 1997. *Humason's animal tissue techniques*, 5th Edition edition. Johns Hopkins University Press, Baltimore, MD.

Tables and figures

Table 1.1. Proportion of age-0 Colorado River cutthroat trout fry surviving from hatching to swimup and their percent daily mortality in the three temperature regimes in Phase 1 of the experiment. The 95% confidence intervals (CI) are shown in parentheses. Survival was significantly lower, and daily mortality was significantly higher, in the intermediate than in the cold and warm regimes, which were not significantly different from one another (see text).

Temperature regime	Days to > 50% swimup	Survival to swimup (95% CI)	Percent daily mortality (95% CI)
Cold	40	0.95 (0.91 - 0.98)	0.14 (0.13 - 0.14)
Intermediate	32	0.91 (0.86 - 0.95)	0.29 (0.24 - 0.32)
Warm	26	0.96 (0.92 - 0.99)	0.17 (0.14 - 0.20)

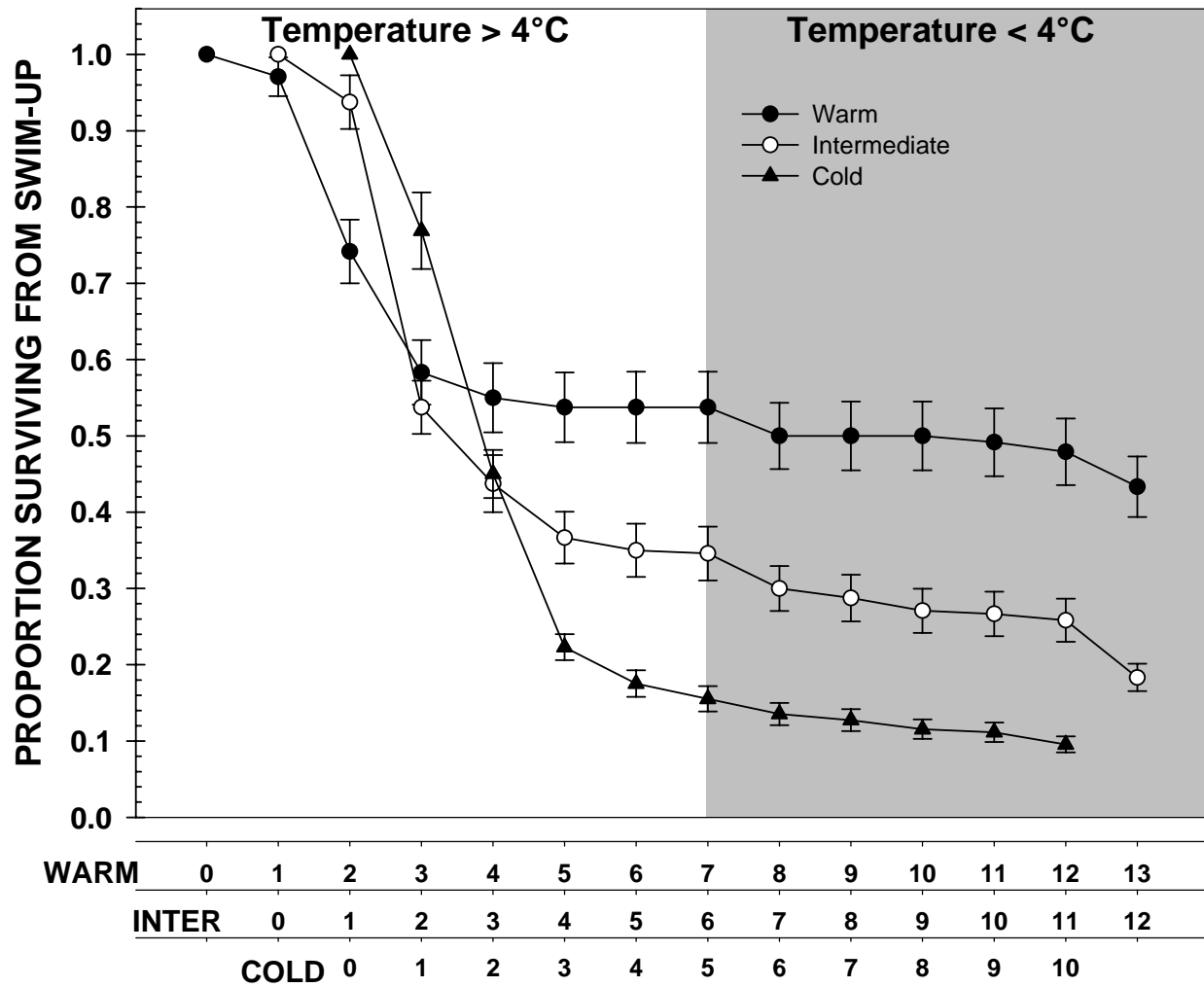


Figure 1.1 Weekly survival for age-0 Colorado River cutthroat trout fry in three thermal regimes created in laboratory streams ($n=8$ for each), from swim-up through 10 to 13 weeks in simulated late summer through winter temperature regimes. Because conditions simulated temperature regimes of natural streams, swim-up and the onset of winter did not occur at the same time in each treatment. The horizontal axes have been aligned so that the onset of winter coincides, defined as the week that temperatures descended below 4°C. Error bars are Kaplan-Meyer 95% confidence intervals.

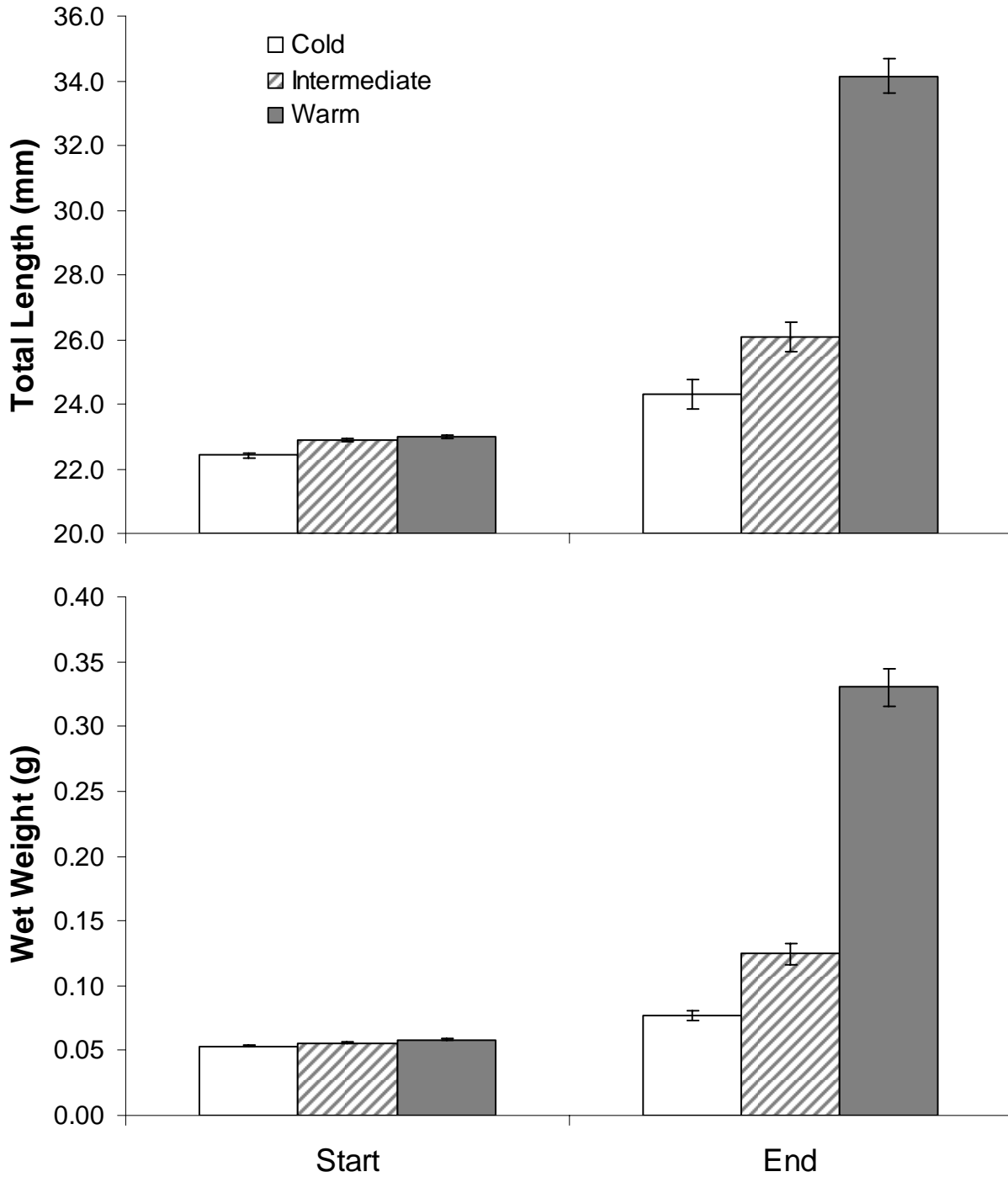


Figure 1.2 Growth expressed in total length (top panel), and wet weight (bottom panel) for Colorado River cutthroat trout fry in three temperature regimes created in laboratory streams from swim-up through 10 to 13 weeks at simulated late summer through winter temperatures (see Figure 1.1). Bars depict 95% Confidence Intervals.

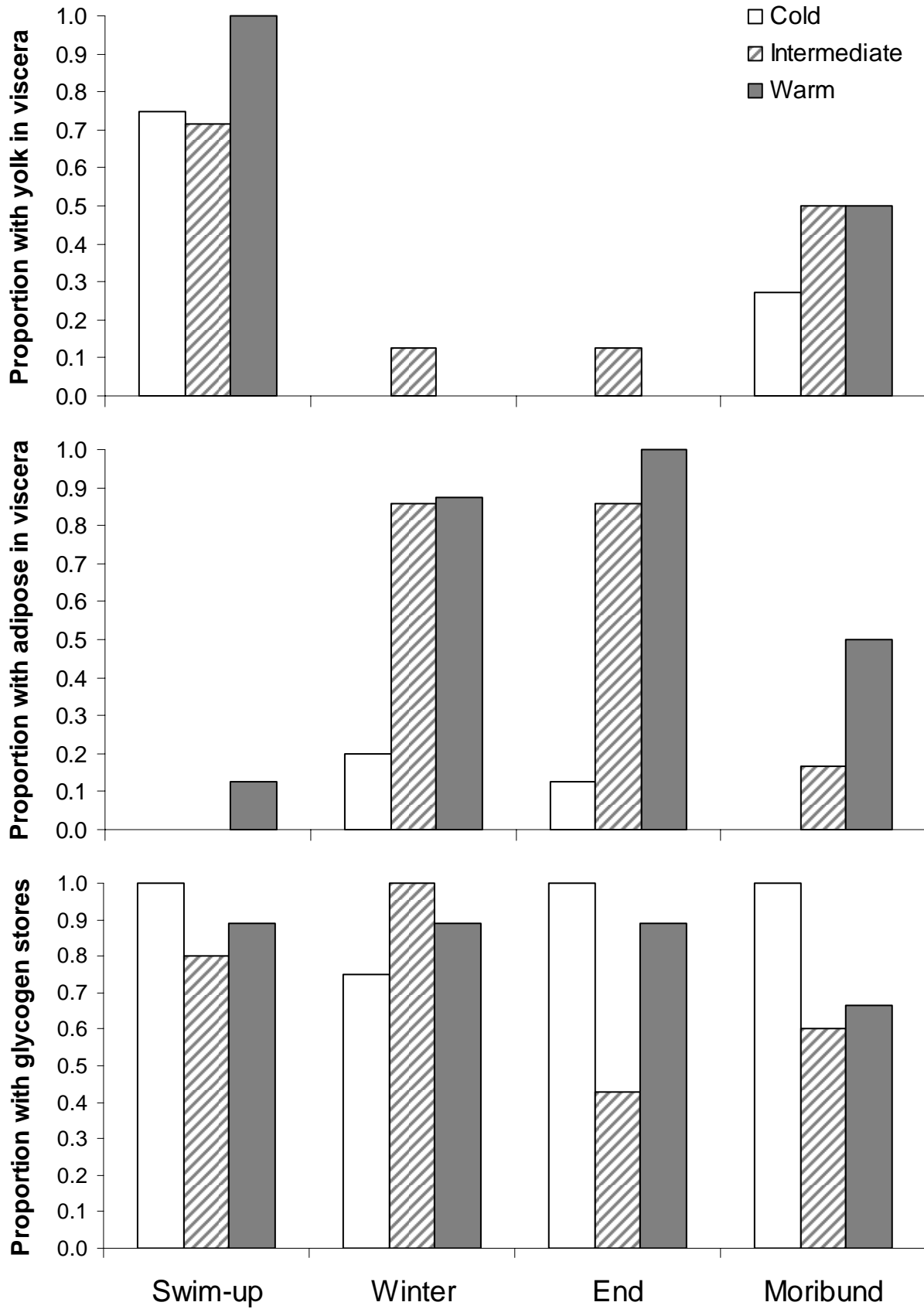


Figure 1.3 Proportion of Colorado River cutthroat trout fry examined during histopathology study that contained at least some visceral yolk (top panel), visceral adipose tissues (middle panel), and glycogen storage vacuoles in liver hepatocytes (bottom panel) in each temperature regime at the start of Phase 2 (Swimup), the onset of winter (Winter), and the end of the experiment (End). An additional set of bars on each panel indicates values for moribund fish examined. These plots do not take into account qualitative differences in these variables noted by the pathologist during analysis (see Appendix A for more detail).

PART 2: COLD SUMMER TEMPERATURE REGIMES CAUSE A RECRUITMENT BOTTLENECK IN AGE-0 COLORADO RIVER CUTTHROAT TROUT REARED IN LABORATORY STREAMS**Abstract**

Translocation of genetically pure fish to create new populations is a key strategy in conservation of three subspecies of native cutthroat trout *Oncorhynchus clarkii* in the southern Rocky Mountains. Previous field research indicated that success of past translocations into high-elevation streams was lowest in those with the coldest summer temperature regimes. We conducted a laboratory experiment to test whether cold summer temperatures can limit translocation success in Colorado River cutthroat trout *O. c. pleuriticus* by hindering recruitment of age-0 fry from hatching through mid-winter. We subjected newly hatched fry to one of three temperature regimes (cold, intermediate, or warm), which averaged 7°C, 8.5°C and 10°C, respectively, during the warmest summer month. The regimes mimicked those of natural streams where translocated fish had died out, or produced populations of cutthroat trout of low or high abundance. During hatching to swimup, mean fry survival rate was high, ranging from 97% in the warm regime to 85% in the cold, and was significantly lower in the cold regime than either the intermediate or warm regimes. After swimup, fry in the warm regime grew more than 60% larger on average than those in the cold regime by the onset of winter. Survival rates were also higher in the warm regime (76%) than in the intermediate (62%) and cold (29%) regimes through mid-winter. Most mortality in all treatments occurred during a recruitment bottleneck during the 4-week period before the onset of winter temperatures. These findings confirm results from a companion field study, and indicate that cold temperatures not only reduce survival

during the first summer, but also reduce growth, which may further reduce chances of survival during winter.

Introduction

In recent years, fisheries biologists have sought to reverse the declines of native cutthroat trout *Oncorhynchus clarkii* in the southern Rocky Mountains, which were driven to low levels by invasions of nonnative salmonids, habitat destruction, and overfishing (Young 1995; Harig et al. 2000). The cutthroat trout is a polytypic species, originally widely distributed in the western U.S. and arranged in 14 subspecies (Behnke 1992, 2002). Three subspecies inhabit the southern Rocky Mountains, Colorado River *O. c. pleuriticus*, greenback *O. c. stomias*, and Rio Grande cutthroat trout *O. c. virginalis*. All three are the subject of conservation management plans aimed at increasing their distribution and abundance, to prevent further endangerment and listing under the Endangered Species Act (USFWS 1998; CRCT Task Force 2001; CDOW 2003). The main strategies to achieve this goal are reconnaissance in remote watersheds to find undiscovered populations of genetically pure native cutthroat trout, and translocation of pure native trout into streams and lakes above barriers where nonnative salmonids have been removed or no trout occurred originally. Translocations are expensive and time-consuming, however, so fisheries biologists in Colorado have sought to understand better the factors determining translocation success in high-elevation streams.

Harig and Fausch (2002) reported that cold summer stream temperature was an important factor limiting translocation success in greenback and Rio Grande cutthroat trout, in conjunction with physical habitat. Translocation success, measured in three categories of adult fish abundance, increased with mean July stream temperatures, bankfull pool width, and the number

of deep pools. Model predictions indicated that translocations would likely fail to establish populations in streams with mean July temperatures $<7.1^{\circ}\text{C}$ (at the average width and number of pools). In otherwise similar streams with mean July temperatures averaging 7.8°C and 10.0°C , the model predicted low and high abundance populations, respectively. State and federal fisheries managers throughout the central and southern Rocky Mountains have recently begun applying the model of Harig and Fausch (2002) to evaluate potential translocation sites and the expected long-term success of current populations in streams (cf., Young and Guenther-Gloss 2004; Young et al. 2005). Although this model is widely used, its accuracy may be limited because it is based on correlative evidence from a modest sample size of streams ($n=27$), of which only six were cases where cutthroat trout died out. Therefore, there is potential for error when predictions are made for other streams if other unmeasured factors that are correlated with July temperature, or habitat variables, actually control translocation success.

Previous research and field data suggest that the mechanism for poor translocation success at cold temperatures may be mortality of age-0 fish during their first few months of life. High-elevation streams where cutthroat trout have been translocated are cold. Most reach only $6-11^{\circ}\text{C}$ during summer when cutthroat trout spawn and their fry feed and grow, and remain near freezing during winter (Harig and Fausch 2002). Cold temperatures are known to reduce survival in eggs of rainbow trout and cutthroat trout (Hubert et al. 1994; Stonecypher et al. 1994; Hubert and Gern 1995b), and decrease metabolic efficiency during egg development (Morrison and Smith 1980; Dwyer et al. 1981). Harig and Fausch speculated that cold temperature regimes also delay emergence and reduce growth of age-0 fry, which may reduce their survival during their first summer or the ensuing winter. However, to date no experiments have been conducted

to measure how cold temperatures affect survival and growth after hatching, particularly using realistic temperature regimes that mimic those in natural streams.

Here we describe the results of a laboratory experiment conducted as part of an integrated laboratory and field study of the effects of cold temperature regimes on translocation success of native cutthroat trout (Coleman and Fausch In review). Our first objective was to test the hypothesis proposed by Harig and Fausch (2002) that cold summer temperatures reduce growth and survival of age-0 cutthroat trout, and may thereby contribute to the failure of translocations in cold streams by limiting recruitment. Our second objective was to characterize the temporal pattern of mortality in different cold temperature regimes to identify any recruitment bottlenecks (i.e., discrete periods of high mortality), which might suggest what physiological or developmental mechanisms are responsible.

Methods

We tested the effect of three natural temperature regimes on growth and survival of age-0 Colorado River cutthroat trout from hatching through mid-winter during a 26-week laboratory experiment that consisted of two phases. The first (Phase 1) encompassed the early larval stage from hatching to swimup, when fish are referred to as ‘sac fry’. The second (Phase 2) encompassed the late-larval and early juvenile stages from swimup through mid-winter. Because translocation success was lower in high-elevation streams in Colorado with colder summer temperatures (Harig and Fausch 2002), we predicted that survival rates would be lower in colder temperature regimes. Further, we predicted that a recruitment bottleneck would occur during Phase 1 or Phase 2 of the experiment, and that the severity of the bottleneck would increase in colder temperature regimes.

Subjects and acclimation.- On 15 July 2003, we obtained eyed eggs of Nanita Lake Colorado River cutthroat trout (Colorado Division of Wildlife, Glenwood Springs Hatchery), a cold-adapted strain, which was originally transplanted from Trappers Lake, Colorado at 2940 m elevation (Behnke 1992). The eggs had been maintained at a constant temperature of 8.8°C for 29 days after spawning. We transported moist eggs in a vented plastic container, resting on ice, to the Foothills Fisheries Facility at Colorado State University, then rehydrated and acclimated the eggs from 8.0°C to 7.0°C over a 30-minute period. We then sanitized the eggs in PVP solution (100 mg/L, Providone iodine in water). Eggs were incubated in three half-full, aerated 40-L aquaria at a constant 7.0°C through hatching, and half the volume of each tank was replaced daily. Upon hatching, 100 sacfry were transferred into each of 24 artificial streams ($n = 2400$) to begin Phase 1 of the experiment on survival rates from hatching to swimup. We acclimated sacfry for 1 hour in plastic bags filled with 7.0°C water from the incubation tanks, due to a 0-3°C difference between incubation and starting temperatures of the experimental regimes (see below).

Temperature regimes.- We synthesized temperature regimes (Figure 2.1) to mimic those of relatively cold high-elevation streams where cutthroat trout translocations had resulted in absent, low, or high abundance populations. We used thermograph data collected by Harig and Fausch (2002) during 1996-1998 from 27 streams studied in the southern Rocky Mountains in Colorado and New Mexico. Temperatures were averaged across streams in each set for each day of the year, and across years, and the results smoothed using a 31-d moving average. To differentiate the regimes evenly based on average summer temperatures, we increased daily temperature in the warm regime to 106% of synthesized values, and decreased daily temperature in the cold regime to 90%. This resulted in three clearly differentiated temperature regimes with

mean August temperatures evenly spaced at approximately 7.0°C, 8.5°C, and 10.0°C.

Thermographs placed near the outlet of one stream in each regime (Onset Tidbit dataloggers, Onset Computer, Bourne, Massachusetts) indicated that mean weekly tank temperatures exceeded those planned only slightly, averaging 0.76°C (SE = 0.06), 0.70 °C (SE = 0.06), and 0.81 (SE = 0.14) higher than planned in the cold, intermediate, and warm regimes, respectively. However, these temperatures should be considered the maxima, because they were collected at tank outlets (downstream end). Temperatures from separate thermographs in the insulated head tanks that provided water to each treatment averaged 0.3 and 0.5°C colder than these values in the intermediate and warm regimes, respectively, but were the same for the cold treatment. Head tank temperatures were used to calculate all temperature variables used in our analyses.

Artificial stream apparatus.- The apparatus consisted of 24 artificial streams (8 for each temperature regime) to which water was supplied at a rate of 4.0 L/min. Each tank was 117 cm long, 51 cm wide, and 25 cm high and had a pea gravel substrate. Screen dividers were used to exclude the fish from turbulence at the inlet and from the standpipe at the outlet, so tank area accessible to fish was approximately 434 cm² (92.5 cm by 49.2 cm). Approximately 116 cm² near the inlet screen was occupied by the outlets of two air lift pumps, which drew water downward through the gravel, provided under-gravel filtration, and generated additional current at the inlet screen. Water depth in each tank ranged from 2.5 cm at the inlet screen to 7.5 cm at the outlet screen. Water velocity in the streams ranged from 1.0 to 7.0 cm/sec, and averaged 2.2 cm/sec when measured at 50% depth at 32 evenly spaced locations in each of two tanks from each regime. Velocities were not different among treatments ($P = 0.68$) or tanks ($P = 0.31$) according to ANOVA with a fixed factor of tank nested within treatment (PROC ANOVA, SAS

v9.1). The tanks were enclosed by insulation and lighted with fluorescent fixtures with 5000 K tubes. Photoperiod was adjusted weekly to mimic the natural photoperiod.

Temperature regimes were generated using a water chilling system and adjustable thermostatic mixing valves. Water chilled by a refrigeration unit and held in a 190 L sump supplied the cold regime treatment, and was mixed with warmer well water to supply the intermediate and warm regime treatments. After use, most water was returned to the sump, but some was diverted to a drain and replaced by pre-chilled well water through a float valve in the sump. Thus, water was shared among treatments and partially recirculated with a continuous fresh water exchange. Tanks were gently cleaned with a siphon weekly to remove excess food and other detritus.

Phase 1.- To determine survival rates of hatchling cutthroat trout fry to swimup in each temperature regime, we placed 100 sacfry within 5 days of hatching in each artificial stream. Sacfry were held in screen boxes (28 cm by 18 cm by 10 cm deep) with opaque covers to shield them from light, prevent burrowing into the gravel, and allow daily mortality counts. We started the experiment at the point in each synthesized temperature regime that matched the number of degree days to which the eggs and fry had already been exposed since spawning (egg take). We estimated the spawning date as the day in each regime that temperatures first exceeded 5°C, because this is the temperature at which native cutthroat trout have been observed to spawn in Colorado (USFWS 1998; B. Rosenlund, U.S. Fish and Wildlife Service, Lakewood, Colorado, personal communication).

During Phase 1, we recorded mortalities daily, and estimated the proportion of fish that had reached swimup. During the first two days, mortalities were assumed to be caused by handling, and were replaced with extra hatchling fry that were maintained at the incubation

temperature (7°C). Phase 1 for each treatment ended when we judged that >50% of sacfry had reached swimup (i.e., the median swimup time). We considered fish to have reached swimup when external signs of remaining yolk were no longer visible, and fish held positions in the water column rather than resting on the bottom. We estimated the proportion of fry reaching swimup after observers remained motionless for 2 minutes, to allow fish to become acclimated to their presence.

Phase 2.- At >50% swimup in each treatment, we selected 20 swimup fry randomly from the basket in each tank, and measured their total lengths (TL) and wet weights. We released these fry into the stream tank and euthanized remaining fry in all but one tank in each treatment. These ‘extra’ fry were used to replace mortalities during the first two days of Phase 2, which we assumed were due to acute effects of handling. Due to the different rates of development in the different temperature regimes, the start of Phase 2 was synchronized to life-history stage (50% swimup) for each treatment, rather than calendar date. As a result, Phase 2 began 14 days later in the cold treatment, and 7 days later in the intermediate treatment, than in the warm treatment (Figure 2.1).

Tanks were inspected daily for mortalities, which were removed and their TL measured, when possible, beginning the fourth week of Phase 2 (dead fry were inadvertently not measured the first three weeks). To measure survival, we carefully counted surviving fish each week. We considered any missing fish as mortalities in our weekly counts, because they could not escape from tanks. A few retreated beneath the gravel and were missed on some counts, but were added to previous counts if they reappeared. We measured the total length and wet weight of each surviving fish at the onset of winter, defined as the week in each regime that temperatures first dropped below 4°C, and at the end of the study, after 14 weeks of simulated winter temperatures.

Fish were measured only three times to minimize handling mortality (only two fish died from handling). We measured the total length of each fish to the nearest 0.5 mm, and wet weight to the nearest 0.0001 g (Scientech analytical balance, model SA-210, Bradford, Massachusetts). We captured each fish in a fine-mesh nylon aquarium net, carefully blotted excess water from the fish and net on a damp natural sponge for 5 seconds, and then transferred the fish into a beaker containing approximately 10 ml of water for which our balance had been tared. At the end of Phase 2, fish were euthanized before weighing by the same procedure.

A minor infestation of the parasite *Ichthyobodo* (formerly *Costia*) was discovered during weeks two through five after swimup (depending on treatment), when mortality increased sharply, particularly in the cold treatment (Mike Minniear and Pete Walker, Fish Pathologists, Colorado Division of Wildlife, personal communication). Within two days of detecting the increased mortalities, we began successful treatment by flushing the artificial streams twice, seven days apart, for 30 minutes with dilute formalin (1:4000), as recommended. There were no acute mortalities during the 48 hours after treatments that could be attributed to the prophylaxis, and a subsequent examination showed that treatment was successful.

Feeding.- When the first fry reached swimup near the end of Phase 1, fish were fed Rangen Trout Starter Diet #0 (Rangen Feeds, Boise, ID) ad libitum once or twice daily. During Phase 2, fish were fed 75% of the optimum ration of Rangen Trout Starter Diet daily using automatic feeders that dropped food particles into the inlet flow continuously to simulate invertebrate drift. The optimum ration was calculated each week based on treatment temperature, fish length, and the number of fish remaining using a commercial feeding chart. We fed 75% rather than 100% of the optimum ration, because cold high-elevation streams are relatively unproductive (Scarnecchia and Bergersen 1986) and the amount of food available to

cutthroat trout fry is likely suboptimal. The swimup fry in our study averaged about 22.5 mm TL, much smaller than the 30 mm minimum on the feeding chart. We were concerned that the minute rations resulting from extrapolation to smaller fish might hinder the development of feeding behavior, so we fed swimup fry the same ration calculated for 30-mm fish.

Data analysis.- For Phase 1, we used one-way analysis of variance (ANOVA) with posthoc least squares means (LSMEANS) comparisons to test for statistical differences in survival among treatments (PROC GLM, SAS v9.1). Such differences could be due to the life stage duration alone (assuming constant daily mortality rate), differences in daily mortality rate, or both (Houde 1987). Therefore, we analyzed arcsine squareroot transformed survival and daily mortality rates (the proportion that died divided by the duration of Phase 1 in days) through >50% swim-up for each treatment.

For Phase 2, we compared the total lengths and wet weights of fish among treatments using a linear model with repeated measures, and used the Satterthwaite method to calculate denominator degrees of freedom (PROC MIXED, SAS v9.1). Because fish were not individually identified, the tank was the experimental unit for this analysis, and the size data were log transformed to correct for the variance increasing with size. Condition indices (i.e., relative weights) were not analyzed, because length-weight relationships are unstable for salmonid age-0 swim-up fry due to morphological changes as fish mature to juvenile stage, and because such indices may not reflect proximate body composition (see Simpkins et al. 2003).

We compared survival rates among treatments for Phase 2 using the normal approximation of the binomial, by calculating Kaplan-Meier 95% confidence intervals (Newman 1995) on survival from swimup to each week of Phase 2. We also fit a logistic-normal model to binomial weekly hazard risk (proportion surviving through each weekly interval) to characterize

the hazard function and estimate random variation due to tank effects (PROC NLMIXED, SAS v9.1). We modeled the hazard function (weekly survival) using accumulated degree days since egg take (DD), dummy variables for treatment (TRT), the DD×TRT interaction, and a random tank effect. In some models, we also included a quadratic term (DD²) and the DD²×TRT interaction, due to an obvious dip in weekly survival rates during the weeks preceding winter that appeared to differ among treatments. Accumulated degree days was chosen as a covariate to integrate both time and temperature in a single variable that is biologically pertinent as an approximation of “physiological time” (Schmidt-Nielsen 1984). We fit four logistic models representing mechanistic hypotheses to determine which covariates best explained variation in the hazard data. These included a full quadratic-logistic model with DD², TRT, and DD×TRT and DD²×TRT interactions, a reduced quadratic-logistic model excluded the TRT effects and interactions, and full and reduced linear-logistic models that were identical to their quadratic counterparts, but excluded the quadratic term (DD²). We used an information-theoretic approach to select the best fitting model based on Akaike’s Information Criteria corrected for small samples size (AIC_c), which allows objectively selecting the model most consistent with the data, while balancing the trade-off between precision and bias (Franklin et al. 2000; Burnham and Anderson 2002). The models were ranked in ascending order based on AIC_c, and the model with the lowest AIC_c was considered the best fitting. Likelihood ratio tests (LRT) were used to distinguish the top model from the other three candidate models.

The best fitting hazard model lacked a hierarchical structure, which made parameter interpretation problematic, and standard posthoc tests for group comparisons were not possible. So, to compare hazard curves among groups, we excluded data from one treatment group at a time, and fit the full (with TRT and interactions) and reduced quadratic models (no TRT or

interactions) to test differences between all three possible treatment pairs. For each group comparison, we used LRT to determine whether models accounting for variation among treatment pairs explained weekly survival significantly better than models that did not. A lower AIC_c for the full model and a significant LRT were taken as evidence of a statistical difference between treatment pairs.

We used a regression approach to determine whether mortality was size-related within each treatment. A model was fit to describe the relationship between time and fry total lengths at swimup, the start of winter, and the end of the experiment. A similar model was fit to the total lengths of fry that died during this period. A third model was fit to the relationship between time and total lengths of all fry (live and dead). The error sums of squares for the three models were used to calculate an approximate F-test to determine whether the curve predicting size of living fry differed from that for fry that died during the experiment (P. Chapman, Department of Statistics, Colorado State University, personal communication).

Results

Phase 1: Hatching to swimup.- Cold temperature regimes reduced survival of cutthroat trout sac fry to swimup, and markedly increased the time required for this life-history stage (Table 2.1). The proportion of sac fry surviving from hatching to swimup differed among treatments (one-way ANOVA: $F = 10.05$; $df = 2, 21$; $P = 0.0009$), and was lower in the cold temperature regime than in the warm (LSMEANS: $P = 0.0003$) and intermediate regimes ($P = 0.004$). The time required for >50% of sac fry to swim up varied by 14 d among temperature regimes and was only about half as long in the warm versus cold treatment. Daily percentage mortality also differed among treatments (one-way ANOVA: $F = 5.14$; $df = 2, 21$; $P = 0.015$)

and was higher in the cold regime than in the warm (LSMEANS: $P = 0.007$) and intermediate regimes ($P = 0.02$). Survival was slightly higher and daily mortality rate lower in the warm vs. intermediate treatment during Phase 1, but the differences were not statistically significant ($P \geq 0.3$).

Phase 2: Swimup through winter.- Cold temperatures strongly reduced growth in age-0 Colorado River cutthroat trout fry during the interval from swimup to the onset of winter temperatures (4°C), and from this point through 14 weeks of simulated winter (Figure 2.2). Fry growth, measured in total length and wet weight, differed among the three temperature regimes (i.e., interactions between temperature regime and period were significant for both; ANOVA: $P < 0.0001$), so we compared sizes separately for each period. Fry lengths and weights differed significantly among the three temperature regimes at the start of winter and the end of the experiment (LSMEANS: $P < 0.0001$ for all comparisons). Growth was highest between swimup and the start of winter, when fish in the warm temperature regime achieved an average length over 60% greater than those in the cold regime. A surprising finding was that surviving fish in all treatments were larger, on average, after 14 weeks of simulated winter than at the start of winter temperatures (LSMEANS: $P < 0.0001$). This apparent growth was not due solely to size-selective mortality because mean total length of survivors at the end of the experiment plus those that died after the onset of winter (see below), were only 0.9-2.5 mm shorter than the mean lengths without these mortalities. The survivors were also significantly longer than mean lengths at the onset of winter ($P < 0.05$ based on non-overlapping confidence intervals). Thus, it is apparent that fish in the cold and intermediate treatments grew in size at temperatures below 4°C when sufficient food was available, similar to Bear Lake Bonneville cutthroat trout (*O. c. utah*; Behnke 1992).

Cold temperatures also reduced survival rates of age-0 Colorado River cutthroat trout fry from swimup to the onset of winter temperatures, and through mid-winter (Figure 2.3). Survival from swimup was lower in the cold treatment by the onset of winter, and was less than half that in the two warmer treatments after 14 weeks at winter temperatures, based on non-overlapping Kaplan-Meier 95% confidence intervals. Moreover, the proportion of fry surviving in the cold treatment dropped rapidly from swimup through the first three weeks of winter, suggesting that this 7-week period posed a strong bottleneck for survival of cutthroat trout fry reared at these cold temperatures. In contrast, the proportion of fry surviving in the intermediate and warm temperature regimes dropped most rapidly during the 4 weeks before the onset of winter, and slowly thereafter during winter.

This period of high mortality was best reflected in the hazard data (i.e., survival from week to week) as a sharp dip in weekly survival during the 8 weeks following swimup in each treatment (Figure 2.4). Our analysis indicated that the full quadratic model fit the data significantly better (LRT: $P > 0.0001$) than each of the three remaining models ($\Delta AIC > 2.0$), and held nearly all of the Akaike weight (Table 2.2). In addition, the two quadratic models also outranked the two linear-logistic models. The presence of DD^2 in the top two models and significant LRT indicates that the dip in weekly survival during the first 8 weeks after swimup was significant, and the presence of TRT and $DD^2 \times TRT$ interactions in the best fitting model indicate that the dip varied among temperature regimes. The random tank effect was both small and did not differ statistically from zero ($t = 0.59$; $df = 23$; $P = 0.56$) in the top model. Our likelihood ratio tests for treatment differences showed that including treatment effects in the full quadratic-logistic model resulted in a significantly better fit than the reduced model including

only main effects of DD and DD² in all three cases (Table 2.3). Thus, all treatment combinations were significantly different ($P < 0.05$).

Size and mortality.- At the time of death, fish that died were almost all smaller than expected compared to their living counterparts (Figure 2.5). Approximate F -tests indicated that this difference in size between fish that died vs. the estimated size of living fish was significant in the cold ($F = 50.37$; $df = 2, 346$; $P < 0.0001$) and intermediate ($F = 10.83$; $df = 2, 364$; $P < 0.0001$), but not in the warm temperature regime. In addition, nearly all fish that died were shorter than the average total length of fish at the start of Phase 2 of the experiment (i.e., at swimup) in their respective treatments.

Discussion

The results of this experiment confirmed our prediction that cold summer temperature regimes, like those of natural streams where past translocations of native cutthroat trout were not successful, strongly reduce growth and survival of age-0 cutthroat trout after swimup during their first summer and winter. Survival from hatching to swimup was high (85% or greater) in all temperature regimes, but our cold temperature regime increased daily mortality rates and delayed development so that by swimup only 4 weeks of growing season remained before the onset of winter, during which temperatures were dropping rapidly. Survival from swimup through midwinter was much lower in the cold than intermediate or warm regimes, even though the mean summer temperatures were spaced evenly at 1.5°C intervals among regimes. The disproportionately high mortality in the cold treatment suggests that, when environmental conditions other than temperature are constant, there is a strong threshold marked by a sharp decline in survival of age-0 cutthroat trout as mean summer temperature drops from 8.5°C to

nearly 7.0°C. Because survival rates in our cold treatment were low (< 30%) by the end of the study, cold summer temperatures are a plausible mechanism to explain the low recruitment reported for cold, high-elevation streams in recent field studies (Harig and Fausch 2002; Peterson et al. 2004; Coleman and Fausch 2005).

Most mortality in all treatments was due to a strong recruitment bottleneck, a discrete period of decreased week-to-week survival rates preceding the onset of winter. Moreover, this bottleneck was worse in the two colder temperature regimes. The hazard analysis showed mortality was greatest during the 4 weeks before the onset of winter, and for 3 weeks afterwards in the cold temperature regime. In the cold temperature regime, swimup occurred closer to the start of winter, and the recruitment bottleneck occurred immediately after swimup. After swimup, a critical period for salmonid larvae occurs when yolk is depleted and fish must find exogenous prey to survive (Houde 1987; Elliott 1989; Pottinger and Mosuwe 1994). Mechanisms invoked to explain mortality during this period include inability to gain territories (Elliott 1990), starvation (Houde 2002), or physiological mechanisms such as increased stress response and decreased immune response (Pottinger and Mosuwe 1994), all of which are known to be affected by temperature. In our experiment, necropsies of fry that had died in the cold treatment during phase 2 indicated that several had yolk remaining inside their body cavity, which had not closed (buttoned up), several weeks after swimup. These fish had nonetheless died of apparent starvation, suggesting that temperatures were too cold to allow fish to metabolize their yolk and develop normally. Thus, cold summer temperature regimes like these are near the physiological threshold for cutthroat trout recruitment, although adult fish can persist in streams with similar temperatures (Coleman and Fausch In review).

Mortality during the recruitment bottleneck, and during the first half of winter, was size-dependent in the two colder temperature regimes, and indicated that fry must likely exceed some size threshold to survive through winter. Most fish that died were smaller than the average size of their living counterparts, and in the two colder regimes many of these died during winter (Figure 2.5). In contrast, in the warm regime nearly all those that survived to the onset of winter also survived to the end of the experiment. The small fish that died may have failed to gain profitable feeding territories (Elliott 1990), or suffered greater physiological effects leading to poor condition due to individual differences in cold tolerance. During an intensive 4-year study of four cutthroat trout populations (Peterson and Fausch 2002; Peterson et al. 2004) no age-0 and few age-1 trout were captured during annual late summer electrofishing of long segments in two cold, high-elevation streams with mean July temperatures of 6.6°C and 6.9°C. However, careful extensive fry sampling in September during the final year revealed small numbers of age-0 trout of 26 mm and 30 mm median length, respectively. In contrast, age-1 fry were relatively abundant in two warmer, mid-elevation streams with mean July temperatures of 12.7°C and 12.4°C, and median lengths of age-0 trout captured during careful surveys in September were 45 mm and 69 mm, respectively. These observations and our own experimental results suggest that age-0 cutthroat trout fry must reach a threshold length of 30-35 mm to survive over winter (Figure 2.5). Thus, in cold temperature regimes, survival to the start of winter is low due to a severe recruitment bottleneck, and the few surviving fry are likely too small to survive over winter when other physical processes may contribute further to mortality.

The parasite infestation (*Ichthyobodo*) affected all treatments due to extensive water sharing, and may have increased mortality after swimup. However, the infestation can only explain a small portion of the mortality observed, because it was discovered quickly and treated

successfully. Further, no acute mortalities occurred within 48 hours after treatment, and the infestation did not recur. Regardless, fry may be more susceptible to parasite irruptions during the critical period (Pottinger and Mosuwe 1994), especially in cold temperatures due to increased stress response or immunosuppression (Einarsdóttir et al. 2000; Watts et al. 2001). Thus, increased susceptibility to parasites and diseases may be one of several proximate mechanisms accounting for increased mortality at cold temperatures.

Our results show that recruitment bottlenecks at cold temperatures can explain the reduced success of native cutthroat trout translocations in cold high-elevation streams in the southern Rocky Mountain region. They indicate that cold temperature regimes do not provide sufficient thermal units for adequate development and growth of fry, which are needed to ensure survival during the subsequent winter. These results are corroborated by a companion field study which showed that growth and recruitment declined markedly in streams with mean August temperatures below 8.5°C, where <900 degree days accumulated during the growing season, on average (Coleman and Fausch In review). Without adequate recruitment, fish populations remain small and are susceptible to extirpation due to environmental fluctuations (Rieman and McIntyre 1993; Caughley 1994; McElhaney et al. 2000). As a result, subspecies of cutthroat trout in this region are likely to be more susceptible to extirpation at high elevations within their ranges (Myers 1998), due to the cold temperature regimes in these marginal habitats. Together, our lab and field studies indicate that locating streams with temperature regimes exceeding the thresholds we identified will be necessary to increase translocation success of native cutthroat trout in southern Rocky Mountain streams.

Acknowledgments

We thank M. Brandt, K. Brewer, C. Coleman, M. Del Tonto, A. Ficke, M. Hopken, S. Hughes, T. Jenson, A. Klein, C. Levi, K. Ross, D. Rouse, and D. Scott, who assisted with data collection. R. Kolecki and P. Schler provided fish and feed used in this experiment, and P. Walker and M. Minniear provided pathology services. P. Chapman and G. White gave advice on statistical analyses. P. Guenther-Gloss, A. Harig, B. Johnson, C. Myrick, B. Rosenlund, E. Wohl, and M. Young provided helpful comments at all phases of this research. Funds to conduct this research were provided by the Colorado Division of Wildlife (CDOW), the U.S. Department of Interior Central Utah Project Completion Act (CUPCA), the U.S.D.A. Forest Service Fish Habitat Relationships Program (FHR), and the National Park Service Water Resources Division (NPS). We thank T. Nesler (CDOW), R. Swanson (CUPCA), G. Eaglin (FHR), D. Winters (FHR), and J. Tilmant (NPS) for administering these funds. M. Coleman was also supported by scholarships from the West Denver Chapter of Trout Unlimited, John C. and Marrietta Peters Scholarship Fund, and Gregory L. Bonham Scholarship fund.

References

- Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6.
- Behnke, R. J. 2002. Trout and salmon of North America. Chanticleer Press, New York.
- Caughley, G. 1994. Directions in conservation biology. *Journal of Animal Ecology* 63:215-244.
- CDOW. 2003. Conservation plan for Rio Grande cutthroat trout in Colorado. Colorado Division of Wildlife, Denver, CO.
- Coleman, M. A., and K. D. Fausch. 2005. Causes of recruitment bottlenecks in translocated cutthroat trout populations: investigation of low temperature effects. Annual report. Colorado Division of Wildlife, Central Utah Project Completion Act Office, U.S.D.A. Forest Service, and National Park Service, Fort Collins, CO.
- Coleman, M. A., and K. D. Fausch. In review. Cold summer temperature limits recruitment of age-0 cutthroat trout in high-elevation Colorado streams. *Transactions of the American Fisheries Society*.
- CRCT Task Force. 2001. Conservation agreement and strategy for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) in the States of Colorado, Utah, and Wyoming. Colorado Division of Wildlife, Fort Collins.
- Dwyer, W. P., C. E. Smith, and R. G. Piper. 1981. Rainbow trout growth efficiency as affected by temperature. United States Department of the Interior, Fish and Wildlife Service, Bozeman.
- Einarsdóttir, I. E., K. J. Nilssen, S. O. Øren, and M. Iversen. 2000. Temperature influence on arctic charr (*Salvelinus alpinus* L.) antibody response to a cellular antigen. *Polar Biology* 23:231-235.
- Elliott, J. M. 1989. The critical-period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. *Journal of Fish Biology* 35:91-98.
- Elliott, J. M. 1990. Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta*. III. The role of territorial behaviour. *Journal of Animal Ecology* 59:803-818.
- Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12:535-551.
- Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management* 20:994-1004.
- Houde, E. D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2:17-29.
- Houde, E. D. 2002. Mortality. Pages 64-87 in L. A. Fuiman, and R. G. Werner, editors. *Fishery science: the unique contributions of early life stages*. Blackwell Science, Oxford, UK.
- Hubert, W. A., and W. A. Gern. 1995. Influence of embryonic stage on survival of cutthroat trout exposed to temperature reduction. *Progressive Fish-Culturist* 57:326-328.
- Hubert, W. A., R. W. Stonecypher, W. A. Gern, and J. Bobbit. 1994. Response of cutthroat trout embryos to reduced incubation temperature at different developmental stages. *Progressive Fish-Culturist* 56:185-187.
- McElhaney, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of Evolutionary Significant Units. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-42.

- Morrison, J. K., and C. E. Smith. 1980. The effect of water temperature on the sexual development and egg quality of rainbow trout broodstock. United States Department of Interior and United States Fish and Wildlife Service, Bozeman.
- Myers, R. A. 1998. When do environment-recruitment correlations work? *Reviews in Fish Biology and Fisheries* 8:285-305.
- Newman, M. C. 1995. *Quantitative methods in aquatic ecotoxicology*. Lewis Publishers, Boca Raton.
- Peterson, D. P., and K. D. Fausch. 2002. Demography, dispersal, and effects of nonnative brook trout on native cutthroat trout in Colorado streams. Final report to Colorado Division of Wildlife, Central Utah Project Completion Act Office, U.S.D.A. Forest Service, and Trout Unlimited, Fort Collins, Colorado.
- Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: Effects of brook trout on native cutthroat trout. *Ecological Applications* 14:754-772.
- Pottinger, T. G., and E. Mosuwe. 1994. The corticosteroidogenic response of brown and rainbow trout alevins and fry to environmental stress during a "critical period." *General and Comparative Endocrinology* 95:350-362.
- Rieman, B. E., and J. D. McIntyre. 1993. Demographic and habitat requirements for conservation of bull trout. U.S. Forest Service, General Technical Report INT-302, Boise, ID.
- Scarnecchia, D. L., and E. P. Bergersen. 1986. Production and habitat of threatened greenback and Colorado River cutthroat trouts in Rocky Mountain headwater streams. *Transactions of the American Fisheries Society* 115:382-391.
- Schmidt-Nielsen, K. 1984. *Scaling: why is animal size so important?* Cambridge Univ. Press, Cambridge, U.K.
- Simpkins, D. G., W. A. Hubert, C. M. del Rio, and D. C. Rule. 2003. Effect of swimming activity on relative weight and body composition of juvenile rainbow trout. *North American Journal of Fisheries Management* 23:283-289.
- Stonecypher, R. W., W. A. Hubert, and W. A. Gern. 1994. Effect of reduced incubation temperatures on survival of trout embryos. *The Progressive Fish-Culturist* 56:180-184.
- USFWS. 1998. Greenback cutthroat trout recovery plan. Region 6, U.S. Fish and Wildlife Service, Denver, Colorado.
- Watts, M., B. L. Munday, and C. M. Burke. 2001. Immune responses of teleost fish. *Australian Veterinary Journal* 79:570-574.
- Young, M. K. 1995. Conservation assessment for inland cutthroat trout. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-256, Fort Collins, CO.
- Young, M. K., and P. M. Guenther-Gloss. 2004. Population characteristics of greenback cutthroat trout in streams: their relation to model predictions and recovery criteria. *North American Journal of Fisheries Management* 24:184-197.
- Young, M. K., P. M. Guenther-Gloss, and A. D. Ficke. 2005. Predicting cutthroat trout (*Oncorhynchus clarki*) abundance in high-elevation streams: revisiting a model of translocation success. *Canadian Journal of Fisheries and Aquatic Sciences*.

Tables and Figures

Table 2.1. Proportion of age-0 Colorado River cutthroat trout fry surviving from hatching to swimup and their percent daily mortality in the three temperature regimes in Phase 1 of the experiment. The 95% confidence intervals (CI) are shown in parentheses. For both measures, the cold regime was significantly different than the other two regimes, which were not significantly different (see text).

Temperature regime	Days to > 50% swimup	Survival to swimup (95% CI)	Percent daily mortality (95% CI)
Cold	31	0.85 (0.79 - 0.90)	0.45 (0.28 - 0.65)
Intermediate	24	0.95 (0.91 - 0.98)	0.19 (0.09 - 0.33)
Warm	17	0.97 (0.94 - 0.99)	0.15 (0.06 - 0.28)

Table 2.2. Logistic regression models of hazard data (weekly survival) as a function of accumulated degree days (DD) and treatment (TRT). Models were ranked in ascending order, based on Akaike's Information Criterion (AIC). The AIC_c is the AIC corrected for small sample size, K is the number of parameters estimated in the model, and ΔAIC_c is the difference in AIC_c between the candidate model and the model with the lowest AIC_c . The Akaike weights (w_i) sum to 1.0. Likelihood ratio tests (LRT) indicate that the top model fit the data significantly better than the three other candidate models. The predictions of the top model (in bold) were plotted in Figure 2.4

Model (included variables)	AIC_c	K	ΔAIC_c	w_i	LRT $P(\chi^2, df)$
Full quadratic (DD, DD ² , TRT, DD×TRT, DD ² ×TRT)	659.8	9	0.0	1.00	--
Reduced quadratic (DD, DD ²)	720.9	3	61.1	0.00	<0.0001 (73.5, 6)
Full linear (DD, TRT, DD×TRT)	738.4	6	78.6	0.00	<0.0001 (84.9, 3)
Reduced linear (DD)	754.2	2	94.4	0.00	<0.0001 (108.9, 7)

Table 2.3. Likelihood ratio tests (LRT) for treatment differences. The full quadratic model includes accumulated degree days (DD), DD^2 , temperature regime (TRT), and the interactions $DD \times TRT$ and $DD^2 \times TRT$. The reduced models include only the DD and the DD^2 covariates. In each row, a lower AIC_c indicates a better fit, and the LRT tests for differences between the full and reduced model. Comparisons of AIC_c among rows are not valid, because the statistics in each row are based on a different subset of the data.

Group comparisons	AIC_c		LRT
	Full quadratic	Reduced quadratic	$P(\chi^2, df)$
Cold vs. Intermediate	474.6	480.7	= 0.007 (12.2, 3)
Cold vs. Warm	452.7	490.1	< 0.001 (43.5, 3)
Intermediate vs. Warm	455.6	465.2	= 0.001 (15.8, 3)

Figure 2.1. The three synthesized temperature regimes used in the experiments, based on mean daily temperatures from streams studied by Harig and Fausch (2002). The regimes correspond to streams where translocations resulted in absent (cold, $n = 6$), low (intermediate, $n = 8$), or high (warm, $n = 13$) abundance cutthroat trout populations (see text). The diamonds show the point in the regime where we estimate fish spawned, and the solid circles show the start of Phase 1 of the experiment, soon after fry hatched. The squares show the start of Phase 2, when $> 50\%$ of fry had reached swimup. The triangles mark the onset of winter for each regime, and the vertical line marks the end of the experiment.

Figure 2.2. A) Total lengths and B) wet weights of fry at swimup, the start of winter when temperatures dropped below 4°C , and at the end of Phase 2, after 14 weeks of winter temperatures. Bars show ± 1 SE, based on raw data, with tank as the experimental unit ($n = 8$ for each treatment). Within each period, bars with the same letter are not significantly different ($P \geq 0.05$) by comparison of least squares means (see text). The difference in total length between the warm and intermediate treatment at swimup was statistically significant ($P = 0.04$), but small (0.65 mm) and biologically insignificant.

Figure 2.3. Proportion of age-0 Colorado River cutthroat trout surviving from swim-up to each week during Phase 2 of the experiment. The survival curves for each regime have been shifted along the horizontal axis to synchronize them based on the timing for onset of winter temperatures. Bars show Kaplan-Meier 95% confidence intervals.

Figure 2.4. Hazard plots showing survival of age-0 Colorado River cutthroat trout fry each week during Phase 2 for the three temperature regimes. The curves have been shifted along the horizontal axis as in Figure 2.3. The lines are logistic regression curves from the best fitting model (see Table 2.2).

Figure 2.5. Total lengths of living and dead fish for each temperature regime during Phase 2. Living fish (filled symbols) were measured three times (swimup, start of winter, and the end of the experiment). Dead fish (open symbols) were measured at their time of death. The relationship shown is the nonlinear best fit to measurements of live fish. The shaded area shows the approximate minimum size threshold for overwinter survival derived from previous field research (Peterson and Fausch 2002; Peterson et al. 2004).

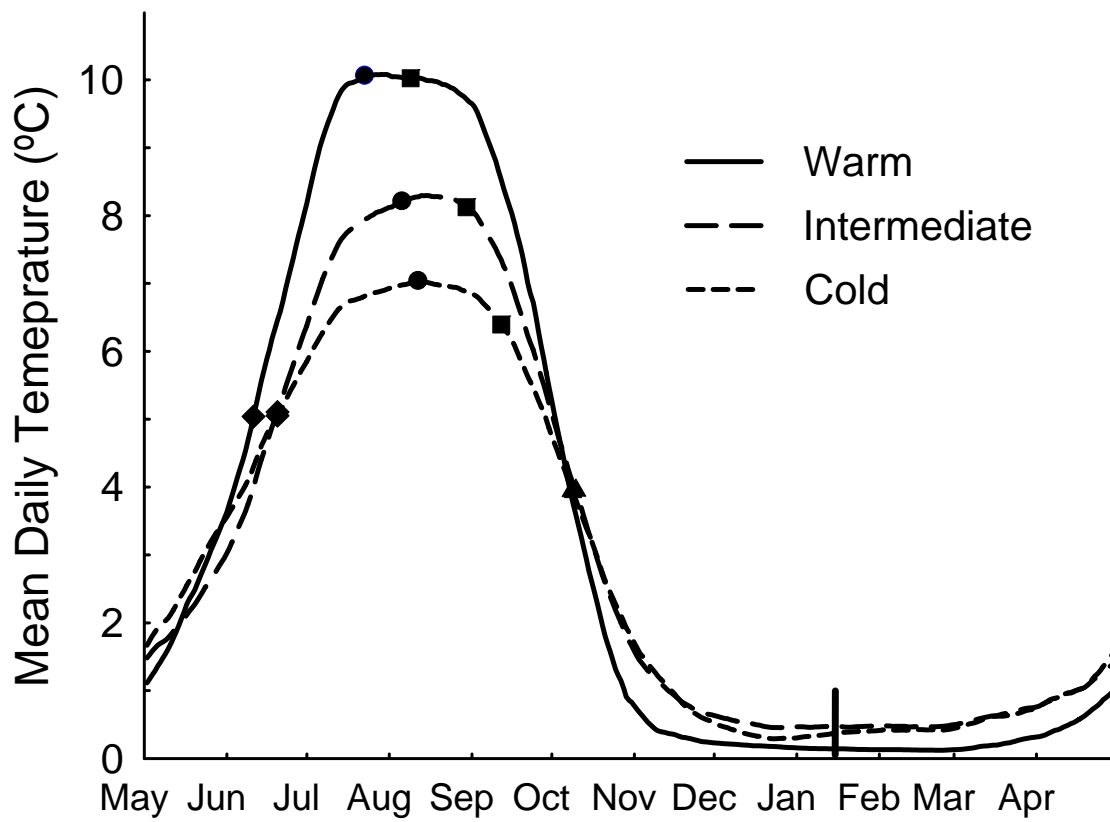


Figure 2.1

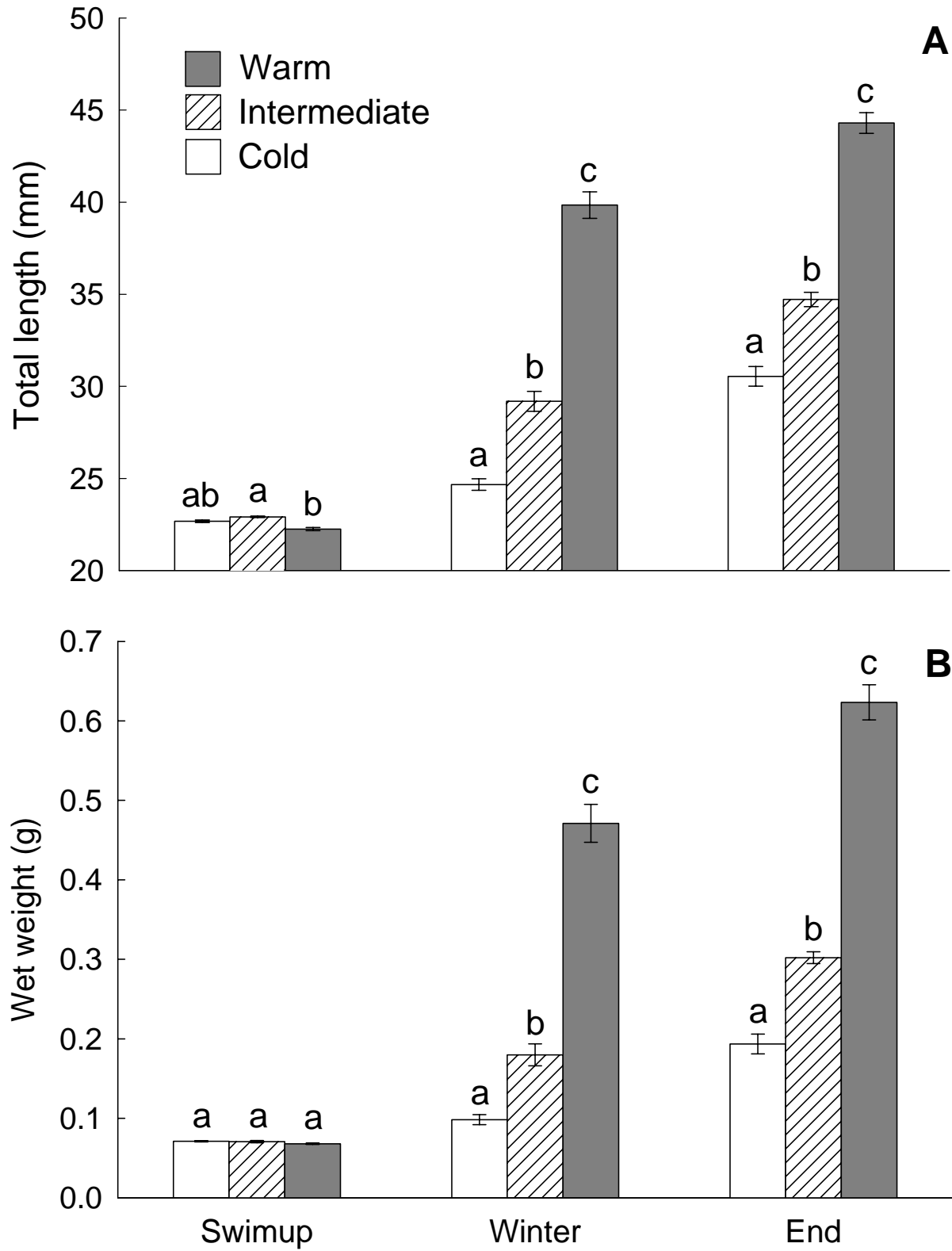


Figure 2.2

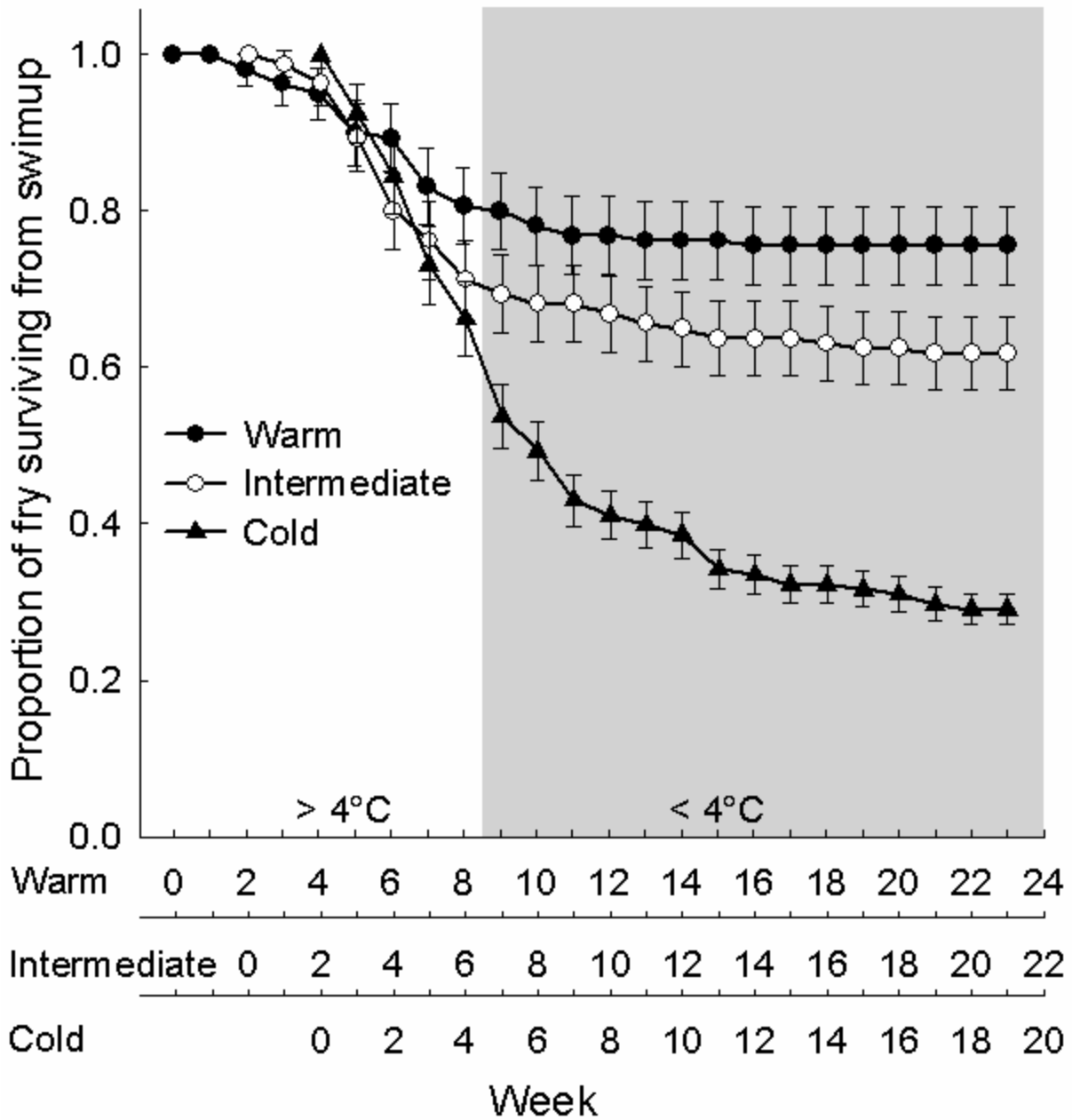


Figure 2.3

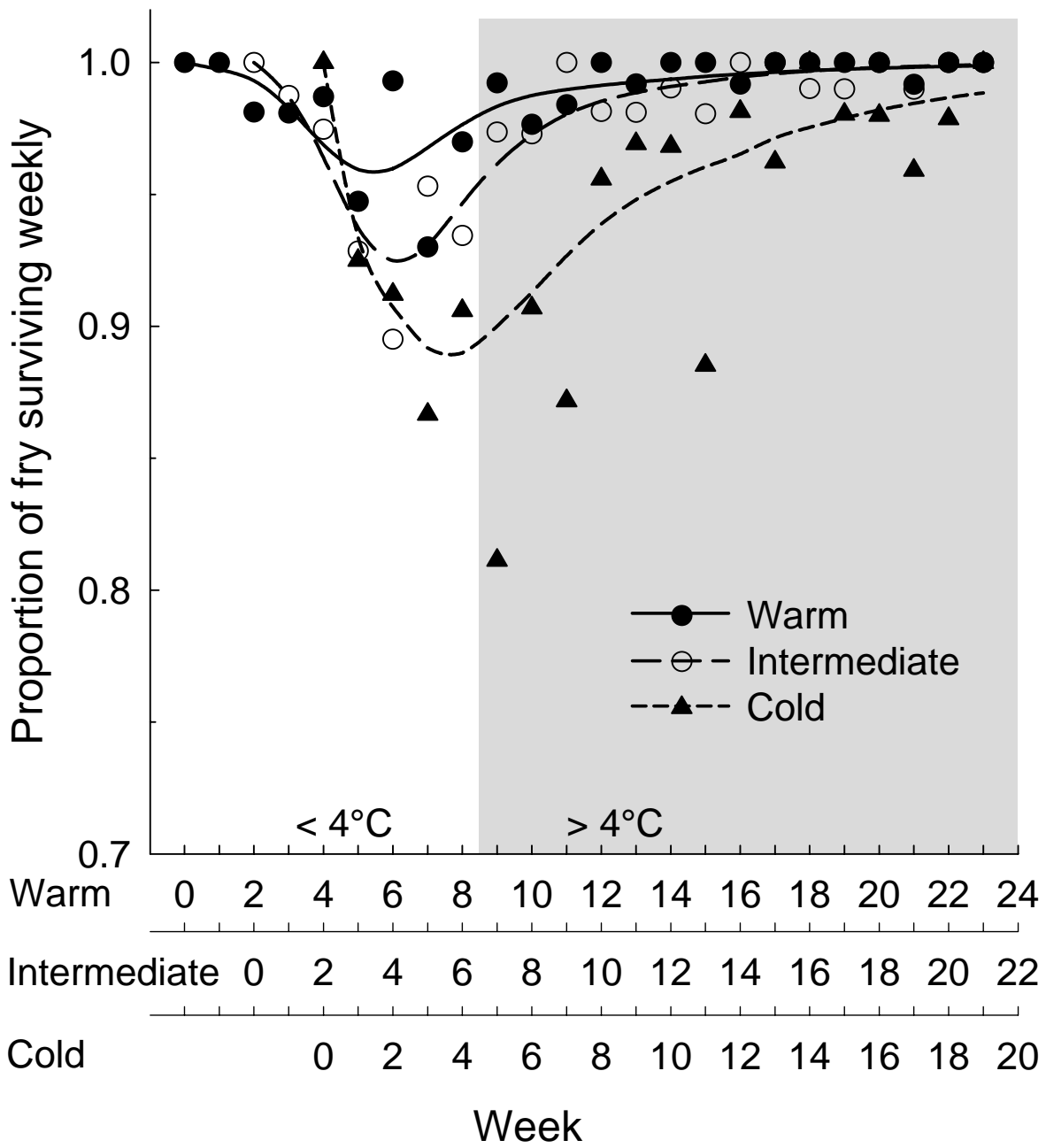


Figure 2.4

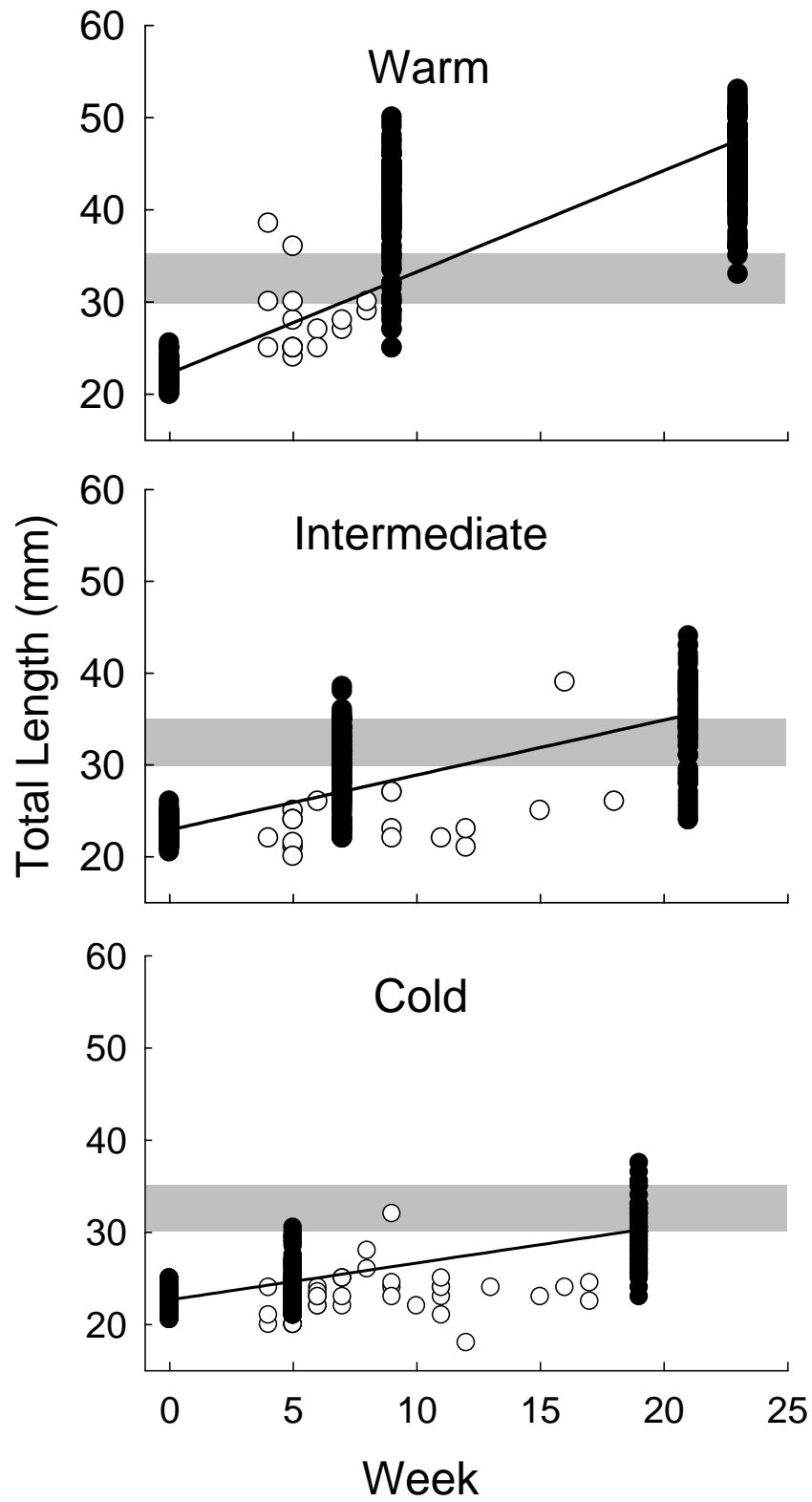


Figure 2.5

PART 3: COLD SUMMER TEMPERATURE LIMITS RECRUITMENT OF AGE-0 CUTTHROAT TROUT IN HIGH-ELEVATION COLORADO STREAMS**Abstract**

Translocation is a key strategy in the management of native cutthroat trout *Oncorhynchus clarkii* subspecies, most of which have declined markedly throughout their historical range. Previous research showed that successful translocations of cutthroat trout in small high-elevation Colorado streams were associated with warm summer water temperatures, and lead to the hypothesis that cold summer temperatures determine translocation success by limiting trout recruitment. We tested this hypothesis in the field by measuring abundance and body size of age-0 cutthroat trout fry (greenback *O. c. stomias* or Colorado River cutthroat trout *O. c. pleuriticus*) in six headwater mountain streams in north central Colorado that varied in thermal characteristics. Fry were measured at peak emergence during three years in two 500-m reaches widely spaced in each stream. Abundance and body size at peak emergence were adjusted using laboratory data to estimate the number and length of age-0 cutthroat trout surviving to the onset of winter. These measures of recruitment and the size of fry at the onset of winter increased with Celsius degree days accumulated during the growing season, and varied among years. A further exploratory analysis showed that spring snowpack and summer precipitation accounted for additional annual variation beyond that explained by temperature. Our results support the hypothesis that recruitment of these subspecies of native cutthroat trout in Colorado can be limited by cold water temperatures that reduce growth and survival. As a general guide, high-elevation streams like those we studied that accumulate 900-1200 Celsius degree days during the growing season afford the best opportunity for recruitment and translocation success. Streams

that provide 700-900 degree days likely sustain recruitment in only some years, and those with less than 700 degree days are unsuitable for translocations due to regular recruitment failure.

Introduction

Introductions to establish or reestablish fish populations, termed ‘translocations,’ are a cornerstone of conservation strategies for rare or endangered species (Griffith et al. 1989; Minckley 1995). One species for which translocations are widely used for conservation is the cutthroat trout *Oncorhynchus clarkii* (Gresswell 1988; Duff 1996). This species is made up of diverse forms widely distributed throughout the coastal and interior western United States, which are arranged in 14 subspecies (Behnke 1992; 2002). Two subspecies are considered extinct, and the rest have declined markedly throughout their native ranges.

Translocations are a key management strategy for the three subspecies of cutthroat trout in Colorado; the greenback cutthroat trout *O. c. stomias* (USFWS 1998), Colorado River cutthroat trout *O. c. pleuriticus* (CRCT Task Force 2001), and Rio Grande cutthroat trout *O. c. virginalis* (RGCT Conservation Plan 2003). The most recent revisions of the management plans for these subspecies call for a total of 401 new translocations, 383 in streams and 18 in lakes. To enhance translocation efforts, fisheries biologists have sought to better understand the mechanisms governing translocation success, and develop tools to help them select streams where the probability of successful translocation is greatest.

Cutthroat trout translocations in Colorado are feasible mainly in isolated segments of high-elevation headwater streams characterized by short, cold growing seasons. Harig and Fausch (2002) analyzed physical habitat variables associated with successful translocations of greenback and Rio Grande cutthroat trout in these high-elevation stream segments. They

reported that summer water temperature was an important predictor of translocation success, and hypothesized that cold summer temperature had detrimental effects on recruitment of age-0 fish. Although adults can survive in these cold thermal regimes, low temperatures may delay development and growth of age-0 cutthroat trout (Hubert et al. 1994; Stonecypher et al. 1994; Hubert and Gern 1995a) and leave them too small to survive the winter (Hunt 1969; Cunjak and Power 1987; Biro et al. 2004). Indeed, a recent laboratory experiment we conducted showed that cold thermal regimes caused a severe recruitment bottleneck near the end of the first growing season, when temperature drops rapidly before the start of winter (Coleman 2005).

Recruitment is a strong determinant of fish population size (Hjort 1914; Houde 1987; Myers 2001), because many factors that influence adult abundance do so through effects on recruitment processes during early life-history stages (Houde 2002). Recruitment is also strongly related to physical conditions during larval development (Elliott 1990; Houde 2002), because in harsh physical environments a large proportion of larval fish may fail to survive to the juvenile stage. This transition from the larval to juvenile stage has been referred to as a “critical period” in development (Hjort 1914; Houde 1987), because it is characterized by a sharp increase in mortality as yolk is depleted and fish must begin to compete for resources such as prey or feeding territories (Houde 1987; Elliott 1994).

The goal of this study was to test the hypothesis of Harig and Fausch (2002) that cold summer temperature regimes in high-elevation streams in Colorado limit recruitment of native cutthroat trout. We studied headwater populations of greenback or Colorado River cutthroat trout in six streams with a range of cold summer thermal regimes. We predicted that under such harsh physical conditions, growth and recruitment to the end of the first growing season would be greater in warmer temperature regimes. We show that laboratory results can be used to adjust

field data to predict growth and recruitment at the onset of winter, which allows developing guidelines for thermal characteristics needed to promote successful translocation of these native cutthroat trout.

Study sites

We selected six first- or second-order headwater streams in the southern Rocky Mountains in north central Colorado for study (Table 3.1). All were located in the Arapaho-Roosevelt National Forest or Rocky Mountain National Park, and contained allopatric populations of either greenback or Colorado River cutthroat trout above barriers to nonnative trout invasion. The streams spanned the range of temperatures for which we predicted recruitment would occur either intermittently and produce a low abundance of adult trout, or consistently and produce high adult trout abundance, based on the model of Harig and Fausch (2002). In each stream, we selected two study reaches near the upstream and downstream end of cutthroat trout distribution that were approximately 500 m long and appeared physically similar. We chose long study reaches to improve the accuracy of our fry counts because fry abundance can be low and their distribution patchy. We spaced reaches as far apart as possible to measure the effects of thermal differences within streams, but excluded low-gradient meadow reaches and beaver ponds. The reaches ranged from 2633-3068 m in elevation at their downstream ends, 2.5-9.0% in gradient, and 3.0-4.4 m in bankfull width. Actual reach lengths ranged from 486-534 m, except in Columbine Creek, a short headwater stream segment where beaver ponds further limited our reaches to 349 and 311 m. Substrates in study reaches were dominated by gravel to cobble sized bed material, and riparian vegetation was dominated by a combination of

Engelmann spruce (*Picea engelmannii*), subalpine fir (*Abies lasiocarpa*), and lodgepole pine (*Pinus contorta*), interspersed with willows (*Salix* spp.).

Methods

We monitored stream temperatures year-round in each study reach, and measured body length and abundance of age-0 cutthroat trout each fall during three years (2002 to 2004). During summer 2004, we also conducted surveys of adult trout and spawning habitat. We predicted age-0 cutthroat trout lengths and recruitment at the onset of winter using field and laboratory data to account for additional growth and mortality after fry surveys. We then related these predicted lengths and recruitment of age-0 cutthroat trout to total growing-season degree days (GSDD; °C). We defined the start of the growing season as the beginning of the first week that average stream temperatures exceeded and remained above 5°C for the season, and the end as the last day of the first week that average stream temperature dropped below 4°C. The GSDD was then determined by summing the daily average stream temperatures over the entire growing season at each study site. These criteria were based on previous work indicating that adult cutthroat trout spawn when stream temperatures reach 5°C (B. Rosenlund and C. Kennedy, US Fish and Wildlife Service [USFWS], unpublished data), and that growth typically occurs in trout at water temperatures approximately 4°C and above (Piper et al. 1982).

Stream temperature monitoring. – We monitored water temperature using two to five thermographs per stream (HOBO Water Temp Pro temperature data loggers, Onset Computer Corporation, Pocasset, ME). During June and July 2002, we deployed thermographs at 13 strategic locations in or near study reaches to measure thermal conditions. Where tributaries entered, we placed additional thermographs downstream to measure their thermal influence. We

placed an additional thermograph in the downstream reach of Cabin Creek in 2003 to measure the effect of a tributary found during the 2002 fry survey. These 14 thermographs measured temperature at 1-hour intervals through the final fry recruitment surveys in September 2004.

Predicting temperature for periods not measured. – Our temperature monitoring began after several weeks of the growing season had elapsed during 2002 and ended several weeks before the end of the growing season in most streams during 2004, so we used local air temperature and precipitation covariates to predict mean weekly stream temperatures for these unmeasured periods. We used segmented linear models to predict stream temperature for each of our thermograph stations (Proc NLIN, SAS v9.1) and conducted a model selection procedure to choose the best model for each site. The segmented regressions fit different conjoined linear relationships for summer and winter data, identifying the best fitting lines for each period based on the predictor variables and the transition point between the two lines (i.e., at the change of seasons). The slope of the line predicting winter temperatures was zero in every case, because winter temperatures stabilize when surface ice forms, but the segmented fit ensured that the lack of an air-water temperature relationship during winter did not detract from the summer fit.

Our primary predictor was local air temperature, with or without a 1-week lag due to the potential for delayed effects on water temperature. We also included two precipitation covariates calculated using data from the National Weather Service Cooperative (NWSC) station nearest each stream that had complete data for 2002-2004. These covariates were spring snowpack measured on May 31 each year, and summer precipitation measured from June 1 to August 31 each year. In some models, the Palmer Drought Severity Index (PDI; Palmer 1965) provided by the Colorado Climate Center (Colorado State University, Fort Collins), which incorporates temperature and rainfall, was used as a covariate instead. In addition, water temperatures

recorded in the same place at different times are not independent, but instead are related to prior water temperatures. Rather than using an autoregressive time-series approach to predict temperatures for periods when we did not have data in 2002 and 2004, we chose to regress water temperature on local air temperature, and accounted for the lack of independence between measurements using one of three covariates. To calculate the covariate values, we used air temperature as a surrogate for stream temperature, because the two are positively correlated (Gu and Li 2002). As covariates, we calculated the difference between the air temperature of the current week (with or without a 1-week lag) and the air temperature of the previous week or the previous two or three weeks. Using this approach, our predictions are not extrapolated, but instead are based on air temperatures that are within the range of those for weeks when we had actual data.

We used an information-theoretic approach to select the “best approximating” segmented linear model using Akaike’s Information Criteria (AIC). This allows objectively selecting the model most consistent with the data, while balancing the trade-off between precision and bias (Franklin et al. 2000; Burnham and Anderson 2002). The model with the lowest AIC_c (AIC corrected for small sample bias) was considered to be the best approximating model among a set of 12 models predicting stream temperature as a function of local air temperature and precipitation covariates or the Palmer Drought Index, for each of 14 thermograph locations. We then used the best approximating models to predict weekly mean water temperatures for the periods during the growing season at each study site not measured in 2002 and 2004.

Surveys of adult trout and spawning habitat. – We conducted visual surveys during July 2004 to determine whether adult cutthroat trout abundance and available spawning habitat was sufficient to sustain recruitment in study reaches. Surveys were conducted early in the growing

season to count fish in or near the areas where they spawn, in case adults emigrate after spawning is complete as previously shown for a population of Colorado River cutthroat trout as well as other salmonids (Young 1996; Northcote 1997; Schmetterling 2001). During summer, cutthroat trout most often inhabit deeper pools (Young 1996), so we adapted visual survey methods used by Harig and Fausch (2002) to estimate adult trout abundance. Two observers wearing polarized sunglasses worked slowly upstream, counting all adult fish (≥ 125 mm) observed in each channel unit. One observer probed beneath undercut banks and around boulders and large woody debris with a wood staff to detect additional fish hiding beneath cover. The other observer held a partly concealed position on the bank and made a second count from this higher vantage point. The two counts were reconciled and recorded. Recent results suggest this method is conservative, detecting 16% of adult abundance estimated by removal electrofishing (Young and Guenther-Gloss 2004). During the same survey we recorded the number of patches of spawning habitat, defined as small areas (≥ 0.5 - 2.0 m²) of clean gravel substrate approximately 0.5-3.0 cm diameter and less than 25% embedded with fine substrate. Most study reaches also had one or more large accumulations of gravel >2.0 m² trapped upstream of boulders or woody debris jams, so we measured the length and width of these patches. Finally, we also compiled data on adult cutthroat trout abundance collected by other researchers in our study streams between 1997 and 2002 (M. Young and P. Guenther-Gloss, US Department of Agriculture Forest Service [USDAFS], unpublished data; B. Rosenlund and C. Kennedy, USFWS, unpublished data; Harig and Fausch 2002).

Fry surveys. – Previous work indicated that cutthroat trout spawn in Colorado mountain streams when mean daily water temperatures reach 5° C in June or July (B. Rosenlund, USFWS, unpublished data), and fry begin to swim-up or emerge from redds after 570-600 Celsius degree

days have accumulated (Coleman 2005), from mid-August through early October. Spawning may occur over two to three weeks once the growing season begins, so 100-200 degree days may have accumulated before some fish spawn. As a result, we predicted that peak fry emergence would occur approximately 600-800 degree days after spawning. We conducted annual surveys to estimate abundance of recently-emerged cutthroat trout fry in each study reach during late August to early October when we estimated this period would occur. In general, we surveyed streams in order from warmest to coldest, based on prior summer temperature data provided by Arapaho and Roosevelt National Forests (K. Sexton, USDAFS, unpublished data), Rocky Mountain National Park (B. Rosenlund and C. Kennedy, USFWS, unpublished data), and previous research (Harig and Fausch 2002). Temperature data we collected during this study (Table 3.2) generally confirmed this order.

Each year, we began surveys after pilot sampling at Roaring Creek, the warmest stream, confirmed that fry had recently emerged and their total lengths were between 20-25 mm. Age-0 cutthroat trout fry less than 30 mm long are weak swimmers and so are confined to small, shallow, sheltered depressions or backwaters with near zero water velocity, which we termed “pockets.” Pockets averaged 0.64 m² (se = 0.02 m²) in area and 0.12 m (se = 0.0014 m) deep (from a random systematic sample of n = 1771 pockets measured in 2002). Nearly all of the pockets were located along channel margins. Pilot surveys showed that newly emerged fry are not easily disturbed by approaching observers, but when startled they seek cover within pockets rather than fleeing into adjacent flowing waters that would wash them away. Because small age-0 fry are confined to pockets, and are relatively easy to find and count compared to larger fry, we were able to use a visual survey method to estimate fry abundance at peak emergence.

Our surveys of fry consisted of pocket-by-pocket visual counts by two observers wearing polarized sunglasses. One observer worked slowly upstream along each bank, counting all age-0 cutthroat trout fry in each pocket encountered. Observers first identified pockets from downstream and conducted a primary visual scan during a careful upstream approach, followed by a close visual count of fry. After the primary count, observers disturbed all available cover in the pocket with a blunt probe. Pocket cover included undercut banks, crevices under and between substrate particles, and any other gaps that small age-0 cutthroat trout fry might enter. This technique sometimes revealed hidden fry, which observers would otherwise have missed. We were able to accurately count fry seen during surveys, because the number of fry per pocket was low (in 2002, 97% contained ≤ 1 fry, $n = 4381$) and observers took care not to suspend silt. Each year, surveyors also captured a sample of up to 10 fry from throughout each reach using fine-meshed dip nets, and measured their total lengths to the nearest 0.5 mm. After processing, we immediately returned fry to their pocket. Fry were not counted in West Creek nor the lower study reach in Roaring Creek during 2003 due to logistical constraints.

Validation of visual fry counts. – Previous research using a careful searching technique for age-0 Colorado River cutthroat trout fry (Bozek and Rahel 1991) indicated that observers saw 78% of the fry (20-52 mm total length [TL]) estimated to be present by three-pass depletion electrofishing, and that their visual counts were significantly correlated with the electrofishing estimates ($r = 0.92$, $P < 0.05$). By comparison, fry we observed during field surveys had emerged recently and were smaller (most 20-35 mm TL), and were confined to pockets due to their weak swimming ability. To determine whether our counts of fry in pockets were accurate, we conducted two trials during July and August 2003 to compare our visual counts to the number of fish captured by electrofishing.

For this validation study, an observer worked upstream, carefully counting fry in pockets as described previously (including disturbing pockets with a blunt probe) and placing flags about 50 cm from pockets to mark them for follow-up electrofishing. The observer was careful to select pockets far enough apart that electrofishing in one would not disturb fry in the next, and did not communicate the results to the electrofishing crew. Fry were not observed to leave pockets, either during or after the visual counts, before electrofishing was conducted. The two-person electrofishing crew, equipped with a backpack electrofishing unit (Coffelt Inc., Model BP-4) operated at 350-400 V at 30 Hz, sampled each pocket within 5 min after the visual counts were conducted. Each pocket was surrounded with dip nets on the offshore side to enclose any fry present (usually 0-2 in each pocket), and workers captured fry during two separate thorough electrofishing passes made to sample all habitat in each pocket. Shocking duration was typically 30-60 sec on each pass, and 60-90 sec elapsed between passes. When observers captured fry on the second pass, they conducted additional passes until no more fry were captured. We summed the total to estimate the number of fry in each pocket.

We evaluated several approaches for calibrating our visual counts using the electrofishing estimates. Regression procedures describing the relationship between two variables that are both subject to measurement error (i.e., Model II regression) were considered, primarily Major Axis Regression (Sokal and Rohlf 1995) because it is most useful when both variables are measured in the same units. However, Major Axis Regression assumes the two variables are continuous and that errors are randomly distributed. In contrast, count data like ours are multinomial and display a Poisson distribution, but there is no established procedure for Model II Poisson regression. In addition, our results indicated that electrofishing sometimes underestimated fry abundance, and so could not be used as a calibration standard (see *Results*). Therefore, we

considered both methods to be indices of true abundance rather than estimates, and used correlation analysis to compare them (Proc CORR, SAS v9.1).

Fry recruitment index. – Although fry were counted at peak emergence, they were surveyed over a 4-6-week period each year, and streams with different temperatures afforded different opportunities for recruitment of fry to the onset of winter. A concurrent laboratory experiment in 2003 (Coleman 2005) that mimicked thermal regimes in high-elevation streams in Colorado indicated that a severe recruitment bottleneck occurs for fry of Colorado River cutthroat trout during the period when temperatures drop between emergence and the onset of winter. Survival rates through the bottleneck were highest in the warmest temperatures tested and lowest in the coldest (7, 8.5, and 10°C mean daily summer temperature). Thus, our fry counts, which were made during the estimated period of peak emergence, may have overestimated recruitment to the onset of winter. Therefore, we used the relationship of fry survival from emergence to the onset of winter as a function of GSDD based on the laboratory data (Figure 3.1) to calculate a fry recruitment index (FRI) for use as a response variable in our statistical analyses. The FRI was defined as:

$$\text{FRI} = F \times S,$$

where: F is the number of fry counted at peak emergence for a given study reach, and S is the probability of subsequent survival from emergence to the onset of winter based on our laboratory data.

Fry size index. – We used fry measurements from both our 2003 laboratory experiment (Coleman 2005) and fry collected in the field during this study to predict the total lengths of fry at the onset of winter (TLW) at each study site, and thereby account for growth between our survey and the onset of winter. Subsequent growth was likely at some study sites where

temperatures remained sufficient for growth several weeks after fry surveys. We planned fry surveys to coincide with peak fry emergence (600-800 degree days after spawning), but survey timing varied somewhat relative to this period so total length measurements in the field were taken over a range of degree days (as were those in the laboratory experiment). This allowed us to fit regression curves that estimated fry total length as a function of accumulated GSDD. An exponential model including GSDD and a class variable specifying lab or field data fit better than a linear model, based on a much lower AIC_c value, so it was used for predictions (Figure 3.2).

Using the exponential model, we defined fry total length at the onset of winter (TLW) as:

$$TLW = \hat{L} + R,$$

where: \hat{L} is the predicted total length of each fry at the onset of winter based on the model, and R is the corresponding residual (actual minus predicted total lengths) for each fry computed from the same exponential regression model. We added the residuals back to the model predictions so that the variance present in our original data was included in the final TLW calculations. This method assumes that each fry grows along a trajectory parallel to the exponential model fit, which is supported by data on salmonid fry size-dependent growth (Post et al. 1999). Simply using the model predictions would have reduced the dataset to a smaller sample of mean values corresponding to the GSDD for each reach and year in which fry were measured ($n=25$ total).

Statistical Analyses and Model Selection. –We performed parallel analyses of the FRI and TLW index values in two stages. The goal of the first stage was to determine the relationships of the fry recruitment index (FRI) and the fry size index (TLW) to GSDD, and whether the index values differed by year. We used an information-theoretic approach to select

the best approximating model (or subset of models) from among a set of three regression models that predicted FRI or TLW as a function of GSDD, year (a class variable), and the GSDD \times year interaction. Growing season degree days was selected as the primary predictor and included in all models, because it theoretically integrates most stream temperature variation that occurs during summer, when eggs and fry are developing. It thus accounts for the most pervasive factor that influences fish physiology and ecology, namely temperature (Brett 1964; Coutant 1976).

Because we found evidence for interannual variation during the first stage of the analysis for both variables, we sought to explain this variation by selecting among several models that included GSDD and precipitation covariates important to stream hydrology. These mechanistic models represented alternate hypotheses to explain interannual variation in FRI and TLW that was not explained by GSDD alone by using hydrologic covariates. The GSDD predictor was again used in all predictive models, and spring snowpack and summer precipitation were the same hydrologic covariates used in our temperature prediction models described above.

Although some hydrologic effects are integrated by our GSDD predictor, spring snowpack and summer precipitation may have additional effects on stream flow, and likely cause fine-scale variation in temperature by altering the rate and direction of hyporheic exchange at spatial scales finer than were detected by our thermographs (Dahm et al. 2003). This is possible because the spatial distribution of low-gradient valley segments where seepage and groundwater exchange are most common in high-elevation streams is patchy.

For both stages of analysis, we developed models predicting FRI using Poisson regression with a log-link function to account for non-normality and heterogeneous variance (Proc GENMOD, SAS v9.1), which is typical in count data. In contrast, we used linear regression models to predict TLW (Proc GLM, SAS v9.1). For both FRI and TLW, we used the

same model selection procedure based on AIC values as for predicting stream temperatures (Franklin et al. 2000; Burnham and Anderson 2002). Models with $\Delta\text{AIC} < 2.0$ were included among the subset of top models. We considered variables important for predicting FRI or TLW values if they occurred in any of the top models, and calculated the relative strength of evidence for each covariate as the sum of the Akaike weights of the models that included the covariate.

Results

Adult trout abundance and spawning habitat. – Data on adult cutthroat trout from all available sources indicated that sufficient numbers were present in our study reaches for successful spawning and recruitment, given favorable conditions (Table 3.2). Our visual surveys during summer 2004 detected 10-54 adult fish in each study reach, although comparison to abundance estimates from removal electrofishing by other investigators suggested that these underestimated abundance by 2-10 times (cf., Young and Guenther-Gloss 2004). We could not conduct adult surveys at West Creek due to high flows, but visual surveys by Harig and Fausch (2002) in 1998 in the same reaches using the same method indicated similar numbers of adults as in our visual surveys of other reaches. Large deep pools in the upper reach likely obscured their visual counts, further underestimating abundance. Overall, the presence of adult fish indicated that adults use habitat in the study reaches during summer, and thus probably spawn there.

Our surveys also indicated that habitat in the study reaches was sufficient for adult trout to spawn (Table 3.2). The density of habitat patches that we deemed potentially suitable for spawning (i.e., $\geq 0.5 \text{ m}^2$) averaged from 2.9 to 14.0 per 100 m, after accounting for reach length. Likewise, the total area of patches $> 2.0 \text{ m}^2$ ranged from 0.0 to 10.4 m^2 per 100 m in the study reaches. Overall, the lower reach of Columbine Creek had the least spawning habitat and the

upper reach of East Fork Sheep Creek had the most. High flows prevented our habitat survey in West Creek during 2004, so we judged the availability of spawning habitat from data collected in 1998 by Harig and Fausch (2002). Their field surveys indicated at least one patch of spawning habitat at the downstream end of each pool, and our field notes described large patches of suitable spawning gravel in the downstream study reach.

Stream temperature monitoring. – Mean August temperature in our study reaches ranged from 6.8 to 9.9°C during the three years of study (Table 3.2). Complete temperature data were measured for August of each year, whereas temperatures were predicted for parts of July 2002 before monitoring began. Nevertheless, temperature was similar for the two months each year. When July mean temperatures for both reaches of each stream were averaged, the temperatures for the three colder streams were < 7.8°C, and for the three warmer streams were > 7.8°C. Harig and Fausch (2002) reported that streams where native cutthroat trout were translocated and achieved low abundance averaged 7.8°C in July, so the study streams spanned the range that they predicted would support low to high abundance of cutthroat trout.

Prediction of stream temperature. – The model best describing stream temperature as a function of air temperature and precipitation covariates for each thermograph location was used to predict temperatures for the first few weeks of summer 2002, before thermographs were deployed, and the last few weeks of summer 2004, after thermographs were retrieved. The best fitting models accounted for most of the variation in measured stream temperature from midsummer 2002 through the fry surveys during fall 2004 ($R^2 = 0.90-0.97$). We combined predicted temperatures with actual data collected during thermograph deployment to estimate the full summer thermal regimes for 2002 and 2004. The total number of weeks for which we predicted temperatures ranged from 4-14 (median = 10) over three years for all but one study

reach in Cabin Creek where we predicted temperature for 26 weeks for one thermograph placed during 2003.

Validation of visual fry surveys. – A comparison of our careful visual survey technique vs. two-pass removal electrofishing showed that the two methods resulted in nearly equivalent counts of recently emerged age-0 fry (20-35 mm TL). Overall, we counted similar numbers of fry using the visual method versus two-pass electrofishing (19 vs. 18 in Cabin Creek, 12 vs. 14 in Roaring Creek). Counts in each pocket were significantly correlated ($r = 0.75$, $P < 0.0001$, $n = 73$ pockets in Cabin Creek; $r = 0.52$, $P < 0.0001$, $n = 50$ pockets in Roaring Creek), but neither method detected all fry found by the other method. Therefore, we chose to use our visual survey technique throughout this study to avoid the risk of harmful effects of electrofishing on trout recruitment (Snyder 2003), and because it was faster and more efficient for making extensive fry surveys over long stream reaches that included large numbers of pockets. Moreover, our results indicated that two-pass electrofishing was not a suitable standard for calibrating our visual survey method. In particular, electrofishing sometimes failed to detect fry that were seen, probably because pockets were partly inaccessible by electrofishing probes due to complex habitat.

Recruitment of age-0 fry. – Cutthroat trout fry were more abundant in the three warmer streams than the three colder streams over the three years of this study (Figure 3.3). We surveyed a total of 10,218 pockets in the six study streams, and found that the number of fry counted at peak emergence averaged 10.1 per 100 m in the three warmer streams, which accumulated more than 750 GSDD, versus 2.1 per 100 m in the three colder streams that averaged fewer than 700 GSDD. Moreover, fry counts varied among years. The highest fry counts in most of the study streams occurred during 2002, a year of record drought and one of

the warmest during the 110 years of record. The lowest fry counts generally occurred during 2003, a year approaching average for precipitation that was nonetheless one of the warmest years on record. The statewide average annual temperatures for Colorado were 7.9°C during 2002 and 2004 (12th and 14th warmest, respectively), and 8.6°C during 2003 (3rd warmest; National Climate Data Center, <http://www.ncdc.noaa.gov>). Annual precipitation averaged 257 mm during 2002 (driest on record), 361 mm during 2003 (ranked 81st), and 434 mm during 2004 (ranked 33rd).

Recruitment of cutthroat trout fry to the onset of winter was higher in warmer stream reaches, and during 2002 than in the other years. The fry recruitment index (FRI), which adjusted counts of newly-emerged fry using laboratory data on mortality to the onset of winter, increased with growing season degree days (Figure 3.4). We assessed the importance of covariates by fitting models that included both GSDD and a year effect, to determine if there were differences among years that could be explained by factors other than thermal regime (Table 3.3). The best fitting model, with the lowest AIC_c, included only GSDD. This model could not be clearly distinguished from the second-ranked model, which contained GSDD and year entered as a class variable ($\Delta\text{AIC}_c = 1.14$), but a year effect is apparent when the data and predicted relationships are plotted (Figure 3.4).

Further analysis showed that variation in cutthroat trout fry recruitment among years is best explained by GSDD alone, and differences in summer precipitation and spring snowpack, when combined in models with GSDD. Year differences alone do not provide a mechanistic explanation for variation in FRI that is not explained by GSDD alone, so we conducted an exploratory analysis to examine the plausibility of these two additional factors derived from *a priori* mechanistic hypotheses. We predicted that the form of precipitation (snow vs. rain) and

its timing might influence fine-scale thermal conditions, habitat, or food for fry in ways that would not be measured by thermographs. To account for these potential hydrologic effects on spawning and recruitment, we fit seven mechanistic models incorporating GSDD and combinations of spring snowpack, summer precipitation, and their interactions with GSDD (Table 3.3). The three models with the lowest AIC_c could not be clearly distinguished (ΔAIC_c for each is < 2.00), so we calculated the relative strengths of the effects in these models by summing the weights of models in which each effect was included (Table 3.4; Burnham and Anderson 2002). Overall, summer precipitation and spring snowpack carried similar weight as hydrologic factors influencing interannual variation in FRI that was not explained by GSDD.

Size of age-0 fry. – Like recruitment, lengths of cutthroat trout fry were greater in warmer stream reaches, and higher during 2002 than the other years. The index of fry total length at onset of winter (TLW), which accounts for growth after fry surveys based on laboratory data, increased with growing season degree days among stream reaches (Figure 3.5). The model structure and covariates considered in our analyses of TLW was identical to that conducted for FRI, including testing mechanistic hypotheses to account for differences among years. For TLW, the model with GSDD, year, and their interaction had the lowest AIC_c and was clearly the best fitting model with virtually all the Akaike weight ($w_i \approx 1.00$; Table 3.5). The model indicated that fry length at onset of winter increased with GSDD, but that this relationship differed among years.

Variation in fry length at the onset of winter was best explained by GSDD and both spring snowpack and summer precipitation. The “global” mechanistic model that included all three variables and the interactions of GSDD with each hydrologic variable had the lowest AIC_c and was also clearly the best fitting model (Table 3.5). This model accounted for virtually all of

the Akaike weight (>99%), so we did not calculate the relative strengths of the effects of the different variables.

Discussion

The results of this study confirm our prediction that size and recruitment of age-0 cutthroat trout at the onset of winter are greater in warmer streams, and lend support to the hypothesis of Harig and Fausch (2002) that translocation success in high-elevation Colorado streams is governed by temperature due to negative effects of cold thermal regimes on recruitment. Overall, these data indicate that streams like those we studied that accumulate < 700 Celsius degree days during the growing season are unsuitable for translocations of native cutthroat trout due to recruitment failures, those reaching about 700-900 degree days can support adequate recruitment in some years, and those with about 900-1200 degree days will be most suitable for translocations.

The results of this field study, when combined with other field data and results of our previous laboratory experiment, suggest that cold summer water temperatures limit recruitment and translocation success via size-dependent effects on survival. Young and Guenther-Gloss (2004) found that abundance of age-1 cutthroat trout was positively correlated with summer water temperature in 12 streams during 1998-1999, which included two of the streams we studied. Peterson and Fausch (2002) reported that recruitment of age-0 cutthroat trout to the onset of winter, and to age-1 the following year, nearly always failed in cold segments of two high-elevation Colorado streams in which fry averaged only 26 and 31 mm TL by early September. In contrast, recruitment occurred consistently in segments of two other streams that were warmer, where fry reached 45 and 68 mm TL by mid-September. Likewise, Coleman

(2005) found that survival of age-0 Colorado River cutthroat trout in laboratory streams was higher to the onset of winter, and much higher to mid-winter, in two warm treatments where fry averaged 29 and 40 mm TL at the onset of winter than in a cold treatment where the survivors averaged only 25 mm TL. These data suggest that cutthroat trout fry need to reach a minimum of 30-35 mm TL at the onset of winter to allow recruitment to age-1. Our field data indicate that this growth potential can be achieved consistently in streams that exceed about 900 degree days during the growing season (Figure 3.5).

A growing body of research supports the view that survival of age-0 fish is often size-dependent (Cunjak and Power 1987; Shuter and Post 1990; Biro et al. 2004), and indicates that a minimum size threshold predicts individual survival and population recruitment rates. A primary mechanism invoked to explain size-dependent survival is fry starvation during the critical period (Hjort 1914; Houde 1987). Size is related to the amount of energy a fish has stored, and can use to survive over winter (Post and Parkinson 2001; Biro et al. 2004). Our field and laboratory data suggest that in warmer streams more age-0 cutthroat trout make a successful transition to exogenous prey after swim-up, and make this transition earlier, thereby gaining an advantage in size and energy stores due to the extra opportunity for growth. This larger size would allow more age-0 fish to avoid starvation during the ensuing winter. If the 30-35 mm TL minimum size threshold proposed above can be validated and generalized among streams, the abundance of age-0 fish reaching this threshold size by the end of the growing season might be a useful index of recruitment for management purposes.

The results of this study indicate that some reaches of high-elevation streams in Colorado can be marginal habitats for native cutthroat trout due to cold temperatures that reduce fry survival and recruitment. Brett (1964) called temperature the “ecological master factor,” and its

pervasive influences in fish ecology are widely recognized (Coutant 1976). Theoretically, abiotic factors such as temperature have their most marked effects on survival of larval fish (Houde 1987), because at this stage fish are not yet forced to compete for food. However, low temperature may also reduce the availability, quality, and abundance of invertebrate prey (Hogg and Williams 1996) required by newly-feeding fry. In either case, in high-elevation streams that approach the boundaries of the thermal niche for cutthroat trout, the effects of low temperature may be relatively strong and explain recruitment rates better than density-dependent survival (Myers 1998).

In addition to temperature, our exploratory analysis of hydrologic variables to explain differences among years suggested that stream flows may also influence recruitment and growth of cutthroat trout fry. In this regard, spring snowpack and summer precipitation are both important variables explaining interannual variation in age-0 fry recruitment and fry size at the onset of winter. The scope of inference about these hydrologic effects is limited based on three years of data, so additional study of the role of hydrologic variables may be necessary to fully understand recruitment processes for cutthroat trout in high elevation streams.

We attempted to conduct fry counts at an equivalent point in each study stream, during the period of peak emergence of cutthroat trout fry. However, fry counts in our warmer streams were generally conducted slightly later relative to predicted peak emergence than those in the colder streams. Thus, counts in our cold streams, made just before the onset of winter, should have been most accurate because all fry were still small and confined to pockets, and fry that emerged later were too small to survive due to declining temperatures. In contrast, fry counts may have been biased low in a few reaches of the warmer streams, because by the time of survey nearly all fry had emerged and some may have left pockets. For example, during field surveys in

2002 some relatively large age-0 fry were observed outside areas we defined as pockets in the warmest reaches of two streams, the lower reach at Columbine Creek and the upper 300 m of the lower study reach at Cabin Creek (above a cooling tributary). Thus, the timing of surveys likely resulted in slightly conservative estimates of recruitment (the FRI index) in the warmer streams, and greater accuracy for the cold streams where it is needed to identify the minimum thresholds for translocation success.

The relationship described here between recruitment and growing season temperatures is consistent with that for translocation success and summer temperature found by Harig and Fausch (2002), and provides further support for the use of empirically derived temperature criteria to determine habitat suitability of potential translocation streams. For example, these investigators reported that streams that supported only low abundance of native cutthroat trout averaged 7.8°C in July, which was the same threshold we found between streams with modest recruitment versus those with little recruitment (Table 3.2, Figure 3.3). In contrast, translocation sites where Harig and Fausch (2002) found the highest abundance of cutthroat trout averaged 10°C in July, a temperature achieved only in our warmest reaches.

Although our results suggest that recruitment is probable in high-elevation streams where at least 900 degree days accumulate during the growing season, spatial variation in temperature along stream segments may increase or reduce the amount of thermally suitable habitat upstream from migration barriers. For example, both our study reaches at Cabin Creek contained tributaries that cooled their lower sections. In the upstream reach, an average of 835 degree days accumulated during the growing season above the tributary, whereas only 621 degree days accumulated downstream. In the downstream reach, 909 degree days accumulated upstream from the tributary, whereas only 692 degree days accumulated downstream. Therefore, spatial

variation in stream temperature due to geomorphic context can create patches of thermally suitable habitat alternating with unsuitable habitat along stream segments. Further, at East Fork Sheep Creek, where our study reaches accumulated only 581 degree days (upstream) and 641 degree days (downstream) during the growing season on average, and would be considered unsuitable habitat, the population is probably maintained by a large source population in a warmer meadow downstream. Young et al. (2005) indicates that occupied stream length strongly predicts cutthroat trout abundance in a wide range of Rocky Mountain headwater streams, so thermal suitability for cutthroat trout translocations should be assessed through basin-wide temperature surveys and monitoring at multiple locations. Monitoring over multiple seasons may also be necessary for the most accurate assessment of thermal suitability, because of annual variability in temperature and precipitation (i.e., spring snowpack, summer rainfall).

Acknowledgments

We thank the many technicians and volunteers without whom these field data could not have been collected. Funds to conduct this research were provided by the Colorado Division of Wildlife (CDOW), the U.S. Department of Interior Central Utah Completion Act (CUPCA), the U.S. Department of Agriculture Forest Service Fish Habitat Relationships Program (FHR), and the National Park Service Water Resources Division (NPS). We thank Tom Nesler (CDOW), Ralph Swanson (CUPCA), Greg Eaglin (FHR), Dave Winters (FHR), and Jim Tilmant (NPS) for administering these funds. M. Coleman was also supported by scholarships from the West Denver Chapter of Trout Unlimited, Peters Scholarship Fund, and Bonham Scholarship Fund.

References

- Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6.
- Behnke, R. J. 2002. Trout and salmon of North America. Chanticleer Press, New York.
- Biro, P. A., A. E. Morton, J. R. Post, and E. A. Parkinson. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 61:1513-1519.
- Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21:1184-1226.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. Springer, New York.
- Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology 63:215-244.
- CDOW. 2003. Conservation plan for Rio Grande cutthroat trout in Colorado. Colorado Division of Wildlife, Denver, CO.
- Coleman, M. A. 2005. Thermal regimes govern recruitment of cutthroat trout in high elevation streams in Colorado. Dissertation. Colorado State University, Fort Collins, Colorado.
- Coleman, M. A., and K. D. Fausch. 2005. Causes of recruitment bottlenecks in translocated cutthroat trout populations: investigation of low temperature effects. Annual report. Colorado Division of Wildlife, Central Utah Project Completion Act Office, U.S.D.A. Forest Service, and National Park Service, Fort Collins, CO.
- Coleman, M. A., and K. D. Fausch. In review. Cold summer temperature limits recruitment of age-0 cutthroat trout in high-elevation Colorado streams. Transactions of the American Fisheries Society.
- Coutant, C. 1976. Thermal effects on fish ecology. Pages 891-896 in Encyclopedia of Environmental Science and Engineering. Gordon and Breach, New York.
- CRCT Task Force. 2001. Conservation agreement and strategy for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) in the States of Colorado, Utah, and Wyoming. Colorado Division of Wildlife, Fort Collins.
- Cunjak, R. A., and G. Power. 1987. The feeding and energetics of stream-resident trout in winter. Journal of Fish Biology 31:493-511.
- Dahm, C. N., M. A. Baker, D. I. Moore, and J. R. Thibault. 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biology 48:1219-1231.
- Duff, D. A., editor. 1996. Conservation assessment for inland cutthroat trout: distribution, status and habitat management implications. U.S. Department of Agriculture Forest Service, Intermountain Region, Ogden, Utah.
- Dwyer, W. P., C. E. Smith, and R. G. Piper. 1981. Rainbow trout growth efficiency as affected by temperature. United States Department of the Interior, Fish and Wildlife Service, Bozeman.
- Einarsdóttir, I. E., K. J. Nilssen, S. O. Øren, and M. Iversen. 2000. Temperature influence on arctic charr (*Salvelinus alpinus* L.) antibody response to a cellular antigen. Polar Biology 23:231-235.
- Elliott, J. M. 1989. The critical-period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. Journal of Fish Biology 35:91-98.

- Elliott, J. M. 1990. Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta*. III. The role of territorial behaviour. *Journal of Animal Ecology* 59:803-818.
- Elliott, J. M. 1994. *Quantitative ecology and the brown trout*. Oxford University Press, Oxford.
- Franklin, A. B., D. R. Anderson, R. J. Gutierrez, and K. P. Burnham. 2000. Climate, habitat quality, and fitness in northern spotted owl population in northwestern California. *Ecological Monographs* 70:539-590.
- Gresswell, R. E. 1988. Status and management of interior stocks of cutthroat trout. American Fisheries Society, Symposium 4. Bethesda, Maryland.
- Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. *Science* 245:477-480.
- Gu, R. R., and Y. Li. 2002. River temperature sensitivity to hydraulic and meteorological parameters. *Journal of Environmental Management* 66:43-56.
- Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12:535-551.
- Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management* 20:994-1004.
- Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe. *Rapports et Process-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer* 20:1-228.
- Hogg, I. D., and D. D. Williams. 1996. Response of stream invertebrates to a global-warming thermal regime: an ecosystem-level manipulation. *Ecology* 77:395-407.
- Houde, E. D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* 2:17-29.
- Houde, E. D. 2002. Mortality. Pages 64-87 in L. A. Fuiman, and R. G. Werner, editors. *Fishery science: the unique contributions of early life stages*. Blackwell Science, Oxford, UK.
- Hubert, W. A., and W. A. Gern. 1995a. Influence of embryonic stage on survival of cutthroat trout embryos to temperature reduction *Progressive Fish-Culturist* 57:326-328.
- Hubert, W. A., and W. A. Gern. 1995b. Influence of embryonic stage on survival of cutthroat trout exposed to temperature reduction. *Progressive Fish-Culturist* 57:326-328.
- Hubert, W. A., R. W. Stonecypher, W. A. Gern, and J. Bobbit. 1994. Response of cutthroat trout embryos to reduced incubation temperature at different developmental stages. *Progressive Fish-Culturist* 56:185-187.
- Hunt, R. L. 1969. Overwinter survival of wild fingerling brook trout in Lawrence Creek, Wisconsin. *Journal of the Fisheries Research Board of Canada* 26:1473-1483.
- McElhaney, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of Evolutionary Significant Units. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-42.
- Minckley, W. L. 1995. Translocation as a tool for conserving imperiled fishes: experiences in the western United States. *Biological Conservation* 72:297-309.
- Morrison, J. K., and C. E. Smith. 1980. The effect of water temperature on the sexual development and egg quality of rainbow trout broodstock. United States Department of Interior and United States Fish and Wildlife Service, Bozeman.
- Myers, R. A. 1998. When do environment-recruitment correlations work? *Reviews in Fish Biology and Fisheries* 8:285-305.

- Myers, R. A. 2001. Stock and recruitment: generalizations about maximum reproductive rate, density dependence, and variability using meta-analytic approaches. *ICES Journal of Marine Science* 58:937-951.
- Newman, M. C. 1995. *Quantitative methods in aquatic ecotoxicology*. Lewis Publishers, Boca Raton.
- Northcote, T. G. 1997. Potamodromy in Salmonidae—living and moving in the fast lane. *North American Journal of Fisheries Management* 17:1029-1045.
- Palmer, W. C. 1965. Meteorological drought. U.S. Department of Commerce Weather Bureau, Research Report No. 45, Washington, D.C.
- Peterson, D. P., and K. D. Fausch. 2002. Demography, dispersal, and effects of nonnative brook trout on native cutthroat trout in Colorado streams. Final report to Colorado Division of Wildlife, Central Utah Project Completion Act Office, U.S.D.A. Forest Service, and Trout Unlimited, Fort Collins, Colorado.
- Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: Effects of brook trout on native cutthroat trout. *Ecological Applications* 14:754-772.
- Piper, R. G., and coauthors. 1982. *Fish hatchery management*. United States Department of the Interior, Fish and Wildlife Service, Washington, D.C.
- Post, J. R., and E. A. Parkinson. 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82:1040-1051.
- Post, J. R., E. A. Parkinson, and N. T. Johnston. 1999. Density-dependent processes in structured fish populations: interaction strengths in whole-lake experiments. *Ecological Monographs* 69:155-175.
- Pottinger, T. G., and E. Mosuwe. 1994. The corticosteroidogenic response of brown and rainbow trout alevins and fry to environmental stress during a "critical period." *General and Comparative Endocrinology* 95:350-362.
- RGCT Conservation Plan. 2003. Conservation plan for Rio Grande cutthroat trout in Colorado. Colorado Division of Wildlife, Denver, Co.
- Rieman, B. E., and J. D. McIntyre. 1993. Demographic and habitat requirements for conservation of bull trout. U.S. Forest Service, General Technical Report INT-302, Boise, ID.
- Scarnecchia, D. L., and E. P. Bergersen. 1986. Production and habitat of threatened greenback and Colorado River cutthroat trouts in Rocky Mountain headwater streams. *Transactions of the American Fisheries Society* 115:382-391.
- Schmetterling, D. A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River drainage, Montana. *North American Journal of Fisheries Management* 21:507-520.
- Schmidt-Nielsen, K. 1984. *Scaling: why is animal size so important?* Cambridge Univ. Press, Cambridge, U.K.
- Shuter, B. J., and J. R. Post. 1990. Climate, population viability and the zoogeography of temperate fishes. *Transactions of the American Fisheries Society* 119:314-336.
- Simpkins, D. G., W. A. Hubert, C. M. del Rio, and D. C. Rule. 2003. Effect of swimming activity on relative weight and body composition of juvenile rainbow trout. *North American Journal of Fisheries Management* 23:283-289.
- Snyder, D. E. 2003. *Electrofishing and its harmful effects on fish*, Information and Technology Report. U.S. Government Printing Office, USGS/BRD/ITR-2003-0002, Denver, Colorado.

- Sokal, R. R., and F. J. Rohlf. 1995. Biometry. W.H. Freeman, New York.
- Stonecypher, R. W., W. A. Hubert, and W. A. Gern. 1994. Effect of reduced incubation temperatures on survival of trout embryos. *The Progressive Fish-Culturist* 56:180-184.
- USFWS. 1998. Greenback cutthroat trout recovery plan. Region 6, U.S. Fish and Wildlife Service, Denver, Colorado.
- Watts, M., B. L. Munday, and C. M. Burke. 2001. Immune responses of teleost fish. *Australian Veterinary Journal* 79:570-574.
- Young, M. K. 1995. Conservation assessment for inland cutthroat trout. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-256, Fort Collins, CO.
- Young, M. K. 1996. Summer movements and habitat use by Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) in small, montane streams. *Canadian Journal of Fisheries and Aquatic Sciences* 53:1403-1408.
- Young, M. K., and P. M. Guenther-Gloss. 2004. Population characteristics of greenback cutthroat trout in streams: their relation to model predictions and recovery criteria. *North American Journal of Fisheries Management* 24:184-197.
- Young, M. K., P. M. Guenther-Gloss, and A. D. Ficke. 2005. Predicting cutthroat trout (*Oncorhynchus clarki*) abundance in high-elevation streams: revisiting a model of translocation success. *Canadian Journal of Fisheries and Aquatic Sciences* 62:2399-2408.
- Young, M. K., R. N. Schmal, T. W. Kohley, and V. G. Leonard. 1996. Conservation status of Colorado River cutthroat trout. U. S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-282, Fort Collins, CO.

Tables and Figures

Table 3.1. Physical characteristics of habitat in survey reaches in the six study streams, listed from warmest to coldest (see Table 3.2). Geographic coordinates (UTM) were measured in the field at the lower ends of study reaches using a handheld global positioning system (GPS). Gradients were calculated from measurements of reach lengths made during 2004, and elevations estimated with a digital elevation model in a geographic information system (ArcMap v9, ESRI) for GPS coordinates taken in the field at the ends of each study reach. Stream bankfull widths were calculated by averaging measurements made at 100-m intervals in the study reaches during 2004.

Stream	CT subspecies ^a	Study reach	Georeference (UTM)	Elevation at downstream end (m)	Gradient (% slope)	Reach Length (m)	Mean bankfull width (m; SE)
Roaring Cr	GBCT	upper	13 T 434831 4511287	2896	9.0	486	3.1; 0.19
		lower	13 T 435497 4509691	2735	6.8	518	4.2; 0.43
Columbine Cr	CRCT	upper	13 T 433036 4448921	2788	2.5	349	3.5; 0.48
		lower	13 T 432360 4448973	2761	5.4	311	3.8; 0.46
Cabin Cr	CRCT	upper	13 S 439319 4426715	3068	7.5	534	3.9; 0.11
		lower	13 S 438096 4426065	2951	5.0	504	4.4; 0.48
Little Vasquez Cr	CRCT	upper	13 S 431787 4414318	3021	4.8	527	3.1; 0.37
		lower	13 S 432153 4415317	2935	3.8	532	3.0; 0.31
West Cr	GBCT	upper	13 T 454102 4479102	2668	8.3	532	4.3; 0.26 ^b
		lower	13 T 454791 4478631	2633	4.8	505	3.8; 0.45 ^b
East Fork Sheep Cr	GBCT	upper	13 T 438697 4498420	3007	6.3	527	3.5; 0.41
		lower	13 T 437936 4499460	2949	5.8	518	3.1; 0.12 ^b

^a Cutthroat trout (CT) subspecies studied were greenback (GBCT) and Colorado River cutthroat trout (CRCT).

^b From data on bankfull width of pools collected by Harig and Fausch (2002), where their survey overlapped our study reach.

Table 3.2. Measurements of adult trout abundance, and habitat and thermal characteristics of survey reaches in the six study streams, listed from warmest to coldest in terms of growing season degree days (GSDD). Previous surveys of adult trout were conducted by the USFWS and NPS in Rocky Mountain National Park, the USDAFS in the Arapaho-Roosevelt National Forest, and by Harig and Fausch (2002) in several streams. Unless otherwise reported, surveys of adult trout, pools, and spawning habitat were conducted during summer 2004.

Stream	Study reach	Counts of adult trout	Previous surveys of adult trout	Pools ≥ 20 cm residual depth	Spawning habitat		Temperature ($^{\circ}$ C)		GSDD
					No. patches ≥ 0.5 m ²	Area (m ²) of patches ≥ 2.0 m ²	Mean July	Mean August	
Roaring Cr	upper	54	530 ^a	12	43	4.8	8.4	8.2	825
	lower	39	390 ^a	17	30	11.5	9.7 ^b	9.6 ^b	996 ^b
Columbine Cr	upper	15	36 ^c	1 ^d	11	4.8	8.3	8.5	771
	lower	11	24 ^c	17	9	0.0	9.5	9.9	957
Cabin Cr	upper	25	110 ^a	25	75	18.9	7.9	7.9	728
	lower	18	100 ^a	20	15	2.2	8.2 ^e	7.9 ^e	801 ^e
Little Vasquez Cr	upper	11	- ^f	19	25	23.0	7.4	7.2	627
	lower	10	- ^f	13	16	41.3	7.4	7.3	678
West Cr	upper	- ^g	6 ^h	10 ^h	10 ^{g,i}	- ^g	7.3	6.9	611
	lower	- ^g	19 ^h	9 ^h	9 ^{g,i}	- ^g	7.8	6.8	675
East Fork Sheep Cr	upper	28	90 ^a , 11 ^h	20	55	54.6	7.1	6.8	581
	lower	13	80 ^a , 14 ^h	32 ^h	40	-	7.1	7.6	641

-
- ^a Extrapolated from USDAFS electrofishing removal estimates during 1999-2000 from where our study reaches overlapped (M. Young, P. Guenther-Gloss, unpublished data)
- ^b Interpolated by averaging data from the upstream and downstream thermographs approximately equidistant from the study reach.
- ^c Extrapolated from USFWS/NPS electrofishing removal abundance estimates in 2002 from where our study reaches overlapped (B. Rosenlund and C. Kennedy, unpublished data).
- ^d A single large beaver pond (~ 5 m wide, ~20 m long) present during the 2004 habitat survey inundated several former pools which may have met our criteria.
- ^e Based partly on backward temperature predictions for the thermograph recording temperature in the upper 300 m of the reach above a tributary (see details in Methods).
- ^f Reports contracted by the USDAFS indicate a consistent presence of adult fish in pools located within our study reaches during 1998-2001.
- ^g Not surveyed due to high flows.
- ^h From data collected by Harig and Fausch (2002) in 1998, where our survey reaches overlapped.
- ⁱ All pools surveyed by Harig and Fausch (2002) had at least 25% clean gravel in the downstream quarter, which indicates presence of at least one spawning patch per pool within our study reaches based on the criteria we used.

Table 3.3. Poisson regression models of the fry recruitment index as a function of thermal and hydrologic covariates and year effects.

Models were ranked in ascending order, based on Akaike's Information Criterion (AIC). The AIC_c is the AIC corrected for small sample size, K is the number of parameters estimated in the model, and ΔAIC_c is the difference in AIC_c between the candidate model and the model with the lowest AIC_c . The Akaike weights (w_i) sum to 1.0. Year was entered as a class variable. The predicted FRI values from the year-effect model shown in bold italics are plotted in Figure 3.4. The relative strength of effects in the mechanistic models shown in bold are presented in Table 3.4.

Model and parameters	AIC_c	K	ΔAIC_c	w_i
Models with growing season degree days (GSDD) and year effect (n = 33)				
GSDD	-46.89	2	0.00	0.63
<i>GSDD, Year</i>	<i>-45.74</i>	<i>4</i>	<i>1.14</i>	<i>0.36</i>
GSDD, Year, (GSDD * Year)	-39.41	6	7.48	0.01
Mechanistic models (n = 33)				
GSDD	-48.67	2	0.00	0.36
GSDD, summer precipitation, (GSDD * summer precipitation)	-47.32	4	1.35	0.18
GSDD, spring snowpack	-47.12	3	1.55	0.17
GSDD, summer precipitation	-46.28	3	2.39	0.11
GSDD, spring snowpack, (GSDD * spring snowpack)	-46.25	4	2.42	0.11
GSDD, summer precipitation, spring snowpack	-44.55	4	4.12	0.05
GSDD, summer precipitation, spring snowpack, (GSDD * summer precipitation), (GSDD * spring snowpack)	-43.03	6	5.64	0.02

Table 3.4. Relative strengths of effects included in the top five mechanistic models of fry recruitment in Table 3.3. The relative strengths of each effect is the sum of the model weights in which it is included. Growing season degree days sums to one because it is included in all models analyzed.

Effect	Strength of effect
GSDD	1.00
summer precipitation	0.36
spring snowpack	0.34
GSDD \times summer precipitation	0.21

Table 3.5. Linear regression models of the predicted total length of fry at the onset of winter (TLW) as a function of thermal and hydrologic covariates and year effects. Models were ranked in ascending order, based on Akaike's information criterion (AIC). The AIC_c is the AIC corrected for small sample size, K is the number of parameters estimated in the model, and ΔAIC_c is the difference between the candidate model and the model with the lowest AIC_c . The Akaike weights (w_i) sum to 1.0. Year is entered as a class variable. The TLW predictions from the year effect model shown in bold italics are plotted in Figure 3.3. The mechanistic model best fitting the TLW index is also shown in bold.

Model and parameters	AIC_c	K	ΔAIC_c	w_i
Models with growing season degree days (GSDD) and year effect (n = 237)				
<i>GSDD, Year, (GSDD * Year)</i>	<i>597.34</i>	<i>6</i>	<i>0</i>	<i>1.00</i>
GSDD, Year	630.03	4	32.69	0.00
GSDD	690.11	2	92.77	0.00
Mechanistic models (n = 237)				
GSDD, summer precipitation, spring snowpack, (GSDD * summer precipitation), (GSDD * spring snowpack)	597.86	6	0.00	1.00
GSDD, summer precipitation, spring snowpack	626.30	4	28.43	0.00
GSDD, spring snowpack	628.92	4	31.06	0.00
GSDD, spring snowpack, (GSDD * spring snowpack)	645.00	4	47.13	0.00
GSDD, summer precipitation, (GSDD * summer precipitation)	657.88	3	60.02	0.00
GSDD, summer precipitation	672.62	3	74.76	0.00
GSDD	690.11	2	92.24	0.00

Figure 3.1. Proportion of newly emerged Colorado River cutthroat trout surviving to winter as a function of growing season degree days for a laboratory experiment (Coleman 2005). The data are proportion of fry surviving to the onset of winter for eight tanks at each of three temperatures (some tanks had identical values). The fitted relationship is a logistic regression model.

Figure 3.2. Total lengths of Colorado River cutthroat trout fry measured in the 2003 laboratory experiment (open circles), and in each reach throughout the field study (filled circles) as a function of estimated degree days accumulated from spawning (field) or egg-take (lab) to the time of measurement. The fitted relationships shown are the predicted lengths of fry in the lab and field, based on an exponential model that included both laboratory and field data (see text).

Figure 3.3. Counts of cutthroat trout fry and average growing season degree days (GSDD) for study streams. Fry counts and GSDD were averaged across study reaches for each stream, and GSDD were averaged over the three years. Fry were not counted in West Creek or the lower study reach at Roaring Creek during 2003 (see text).

Figure 3.4. Fry recruitment index (FRI) as a function of growing season degree days for the three study years. Fry recruitment index values are the estimated number of fry surviving to onset of winter based on the number of newly-emerged fry counted. The lines plotted are from the best fitting model including a year effect, shown in Table 3.3.

Figure 3.5. Predicted total length of cutthroat trout fry at the onset of winter (TLW) as a function of growing season degree days for the three study years. Each point represents the predicted total length at the onset of winter of fish measured during our fry surveys, and the lines plotted are the linear best fits.

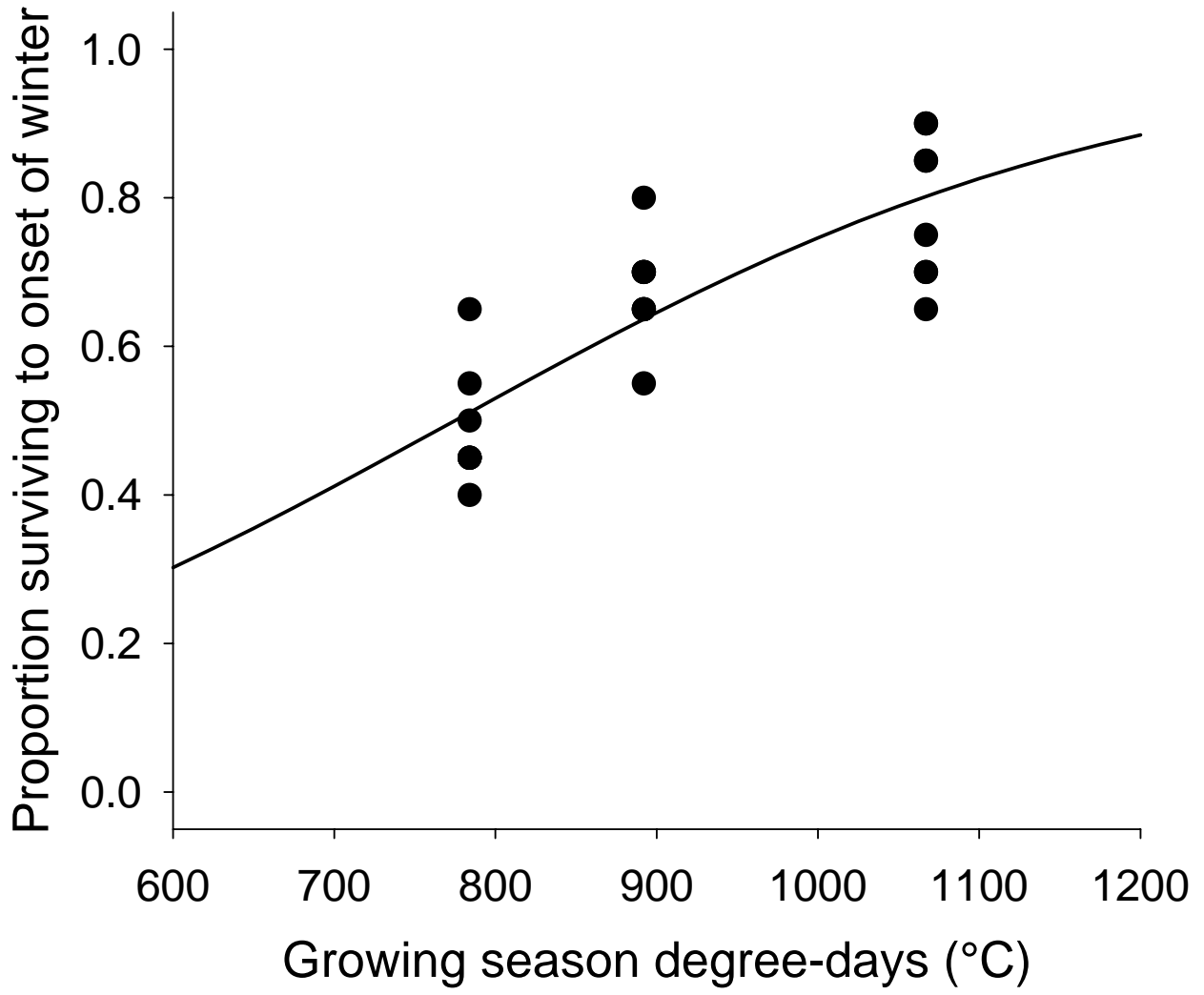


Figure 3.1.

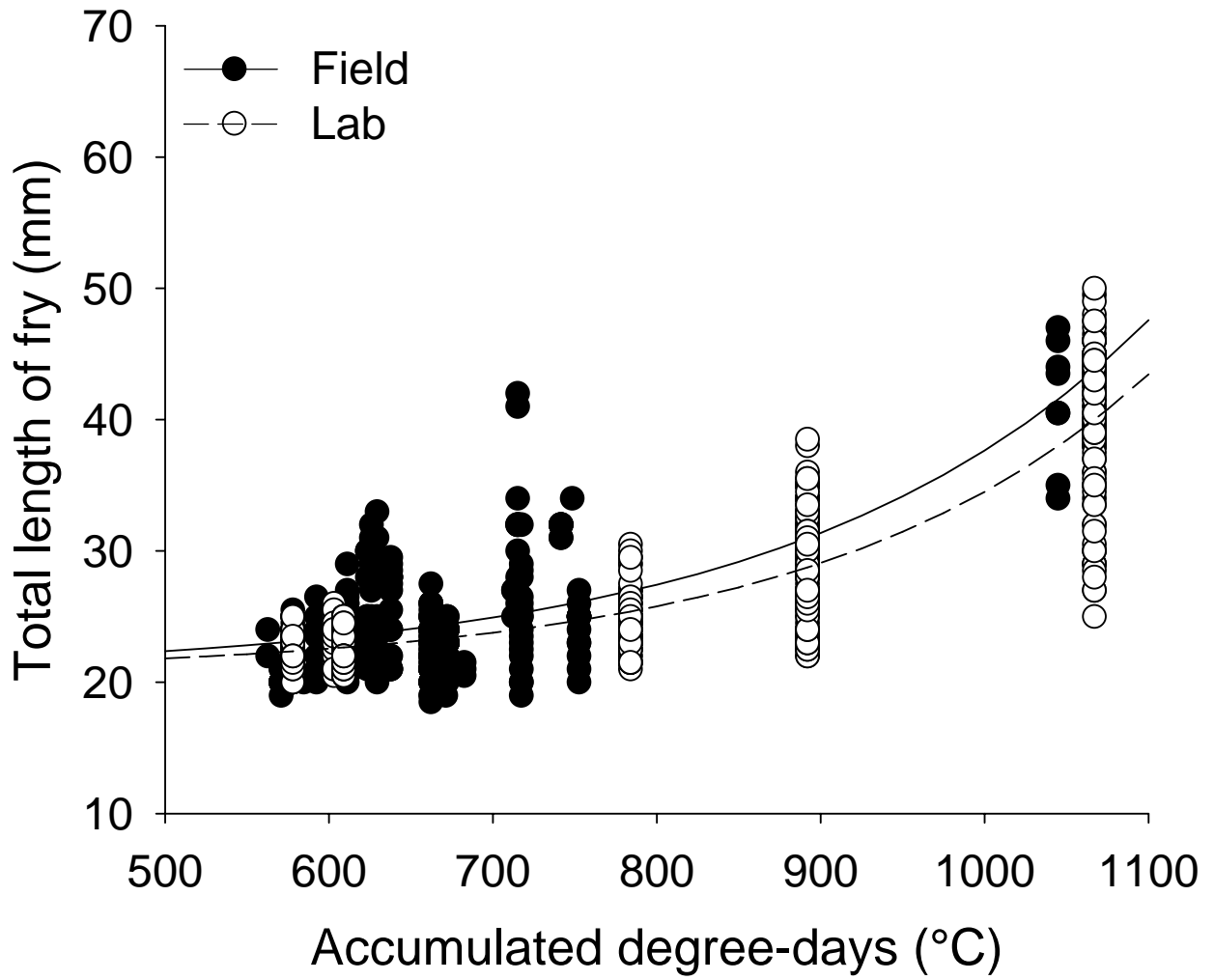


Figure 3.2.

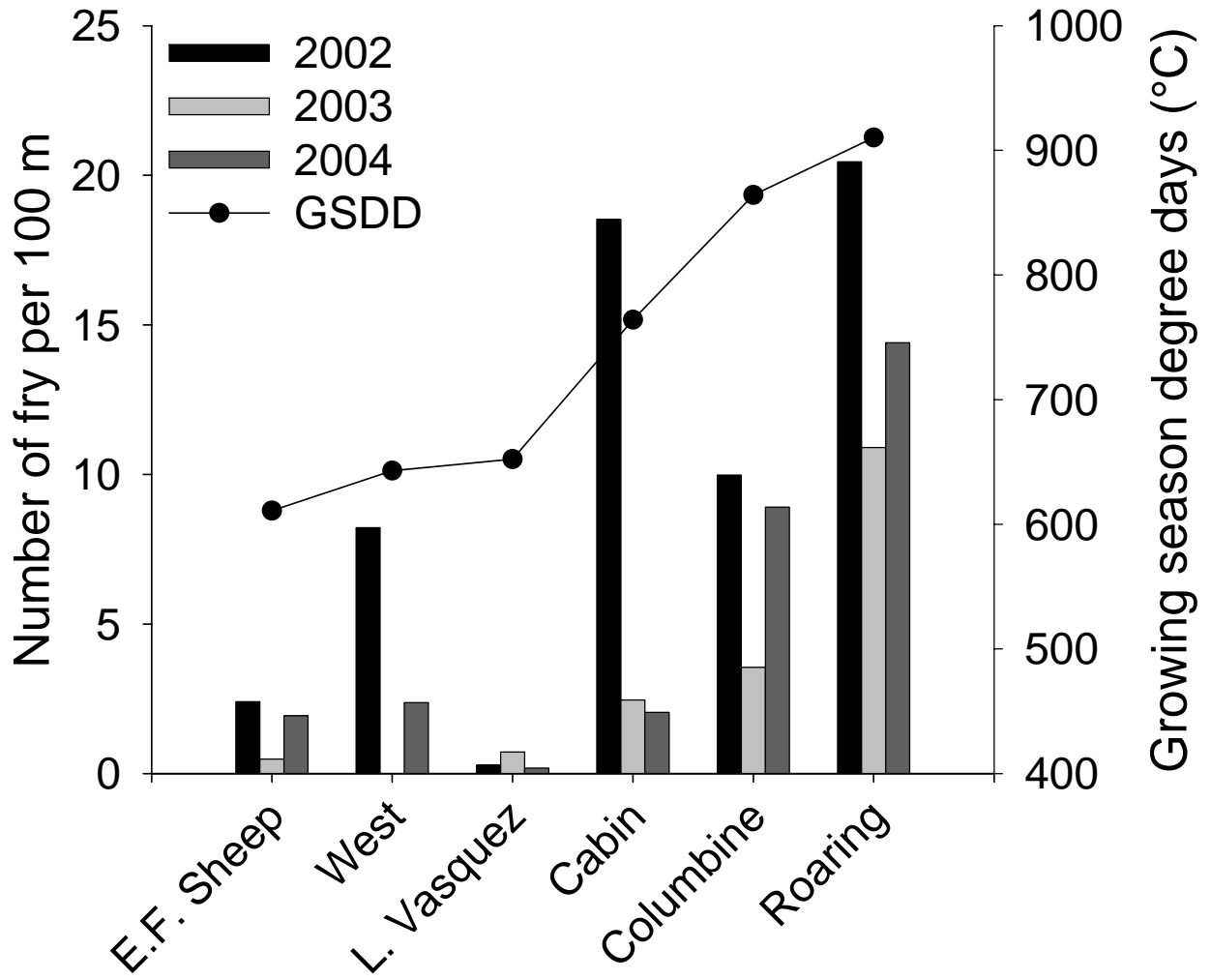


Figure 3.3.

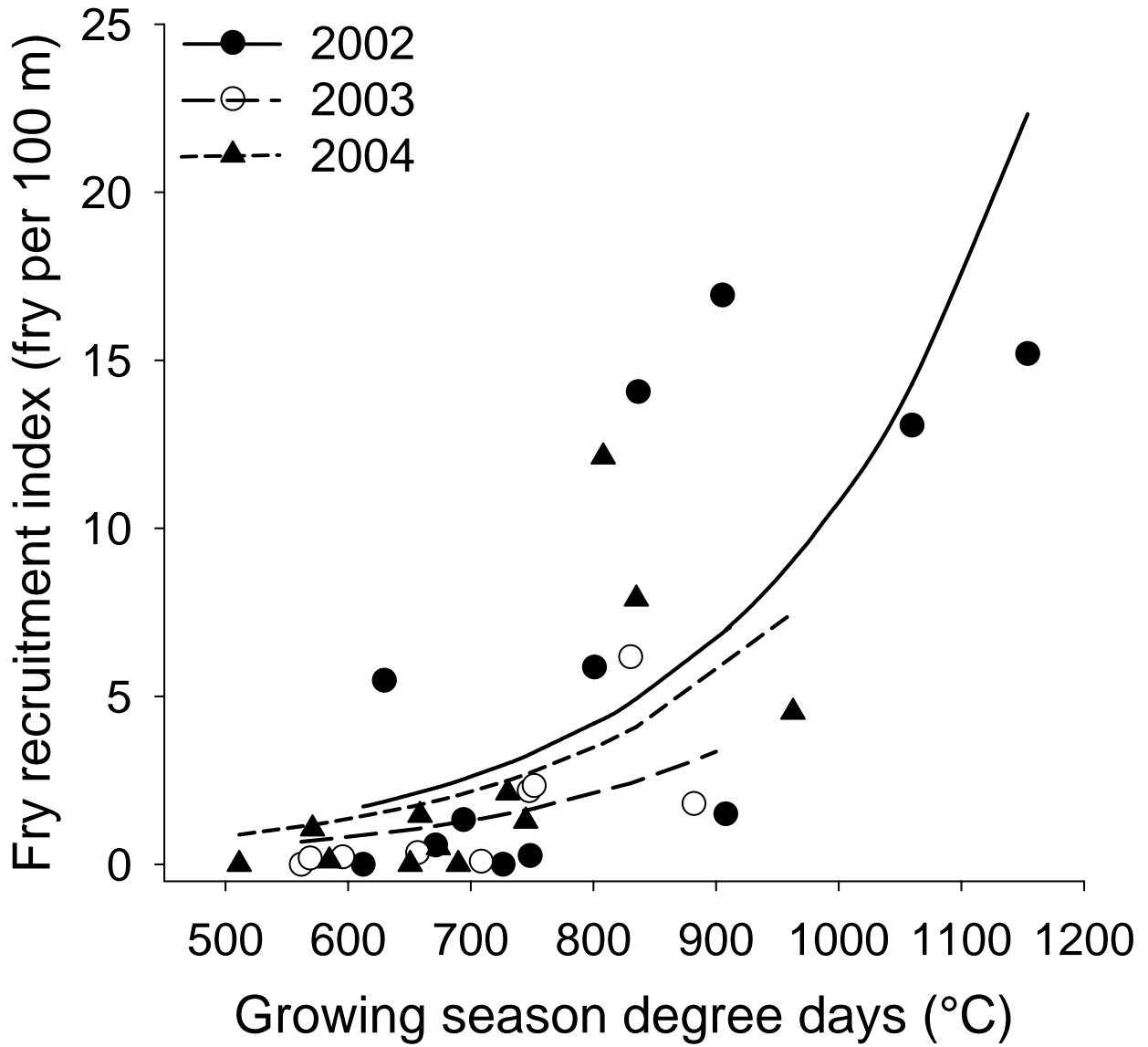
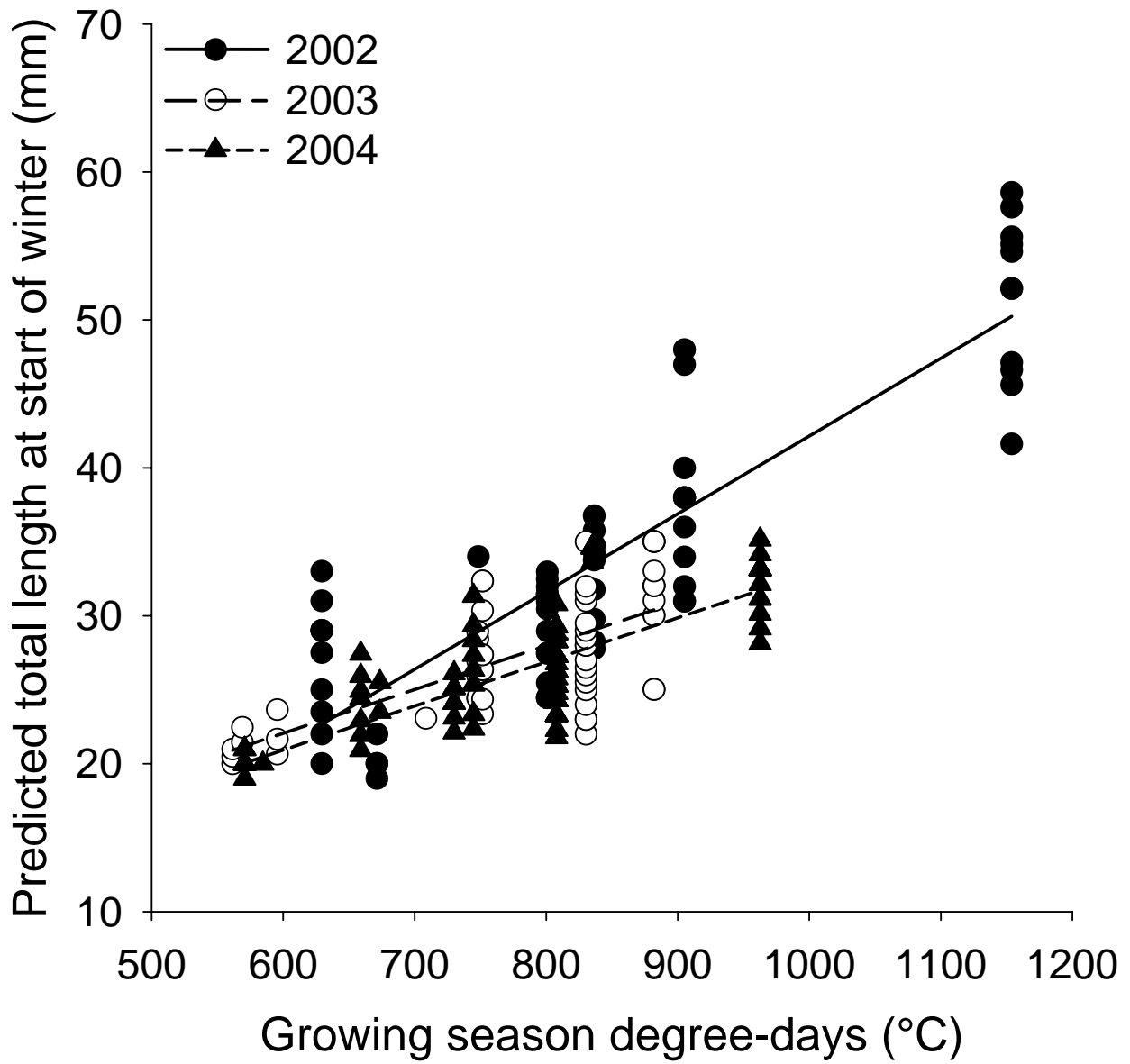


Figure 3.4.



APPENDIX A. HISTOPATHOLOGY STUDY

Charlie E. Smith (US Fish and Wildlife Service, Retired, 212 Story Hill Rd, Bozeman, MT 59715) conducted a histopathological analysis on fish sampled from our laboratory experiment during 2004 (Part 1 of this report and Coleman and Fausch In review). Following is the summary of observations he provided us on December 18, 2005.

HISTOPATHOLOGY EXAMINATIONS

- Charlie E. Smith

Results, Yolk in Visceral Cavity:

As expected, with the exception of swim-up fry, yolk was lacking, in visceral cavities of most of the fish held at warm, intermediate and cold water temperatures suggesting it had been used as a source of nutrients. There was a small amount of yolk in two of four moribund fish held in warm water and three of six fish held in the Intermediate water temperature (figure 1). This finding was similar in fry held in cold water (figure 2). However, a high percentage of moribund fish held in cold water lacked yolk in their visceral cavity suggesting perhaps lack of nutrition was a factor in their death.

Warm Water, Final - there was no apparent yolk in visceral cavities of any fish.

Warm Water, Moribund – two of four fish examined contained small amounts of yolk in their visceral cavity. Yolk was absent in the visceral cavities of the other two fish.

Warm Water, Swim Up – all fish examined contained mild to moderate amounts of yolk material in their visceral cavities.

Warm Water, WTR – all fish lacked apparent yolk material in visceral cavity.

Intermediate Water, Final - yolk was apparent in visceral cavities of only one of 8 fish examined.

Intermediate Water, Moribund – yolk was apparent in visceral cavities of three of six fish examined, but was lacking in the other three.

Intermediate Water, Swim Up – yolk was present in visceral cavities of five fish, but lacking in two.

Intermediate Water, WTR – yolk was present in visceral cavity of only one fish but lacking 7 fish.

Cold Water, Final – yolk was absent in visceral cavities of all fish examined.

Cold Water, Moribund - yolk material was absent in visceral cavities of eight fish, but present in the other three fish.

Cold Water, Swim Up – yolk material was absent in two fish, but present in small amounts in the remaining six fish.

Cold Water, WTR – Yolk material was absent in visceral cavities of all five fish examined.

Results, Adipose in Visceral Cavity:

Visceral fat, with the exception of moribund fish was present in most fish held in the warm and intermediate water temperatures (figures 3 & 4). However, the majority of fish held in cold water lacked visceral demonstrating poor energy reserves.

Warm Water, Final - all eight fish contained mild to moderate amounts of visceral fat.

Warm Water, Moribund – two fish lacked visceral fat and two had mild to moderate amounts.

Warm Water, Swim Up – seven fish lacked visceral fat and one had only a small amount.

Warm Water, WTR – seven fish had small amounts of visceral fat and one fish lacked visceral fat.

Intermediate Water, Final – one fish lacked visceral fat and the other six contained small amounts.

Intermediate Water, Moribund – five fish lacked visceral fat and one contained a small amount.

Intermediate Water, Swim- up – all seven fish lacked visceral fat.

Intermediate Water, WTR - one fish lacked visceral fat and six contained small amounts.

Cold Water, Final – seven fish lacked visceral fat and one had a small amount.

Cold Water, Moribund - all 11 fish lacked visceral fat.

Cold Water, Swim – Up – all eight fish lacked visceral fat.

Cold Water, WTR – visceral fat was lacking in four fish and present in a small amount in one fish.

Results, Glycogen/Fat (g/f) in Hepatocytes:

Glycogen and fat are normally stored in the cytoplasm of hepatocytes (liver cells) of healthy fish. Thus, vacuolation of hepatocytes is indicative of glycogen/fat (g/f) storage, both of which are

totally or partially, respectively, removed during processing for histology. Normally, mild to moderate amounts of g/f are found in hepatocytes of well fed trout. The amount of g/f in hepatocytes varied somewhat with water temperature. However, it was more prevalent in hepatocytes of fish held in warm water, and present in much lesser amounts, in fish held in the cold water. It was absent in the majority of moribund fish held in cold water. (figure 5).

Warm Water, Final –one fish lacked g/f and 8 fish contained mild to moderate amounts of g/f in hepatocytes.

Warm Water, Moribund - one fish lacked g/f, and two had mild amounts of g/f.

Warm Water, Swim – Up –one fish lacked g/f, and eight fish had mild to moderate amounts of g/f in hepatocytes.

Warm Water, WTR – one fish lacked g/f, and eight contained mild to moderate amounts of g/f.

Intermediate Water, Final – four fish lacked g/f, and three contained mild to moderate amounts of g/f.

Intermediate Water, Moribund – two lacked g/f, three fish had mild to moderate amounts of g/f. Two fish had **mild necrosis** of hepatocytes.

Intermediate Water, Swim – Up – one fish lacked g/f, and four had mild to moderate g/f.

Intermediate Water, WTR – seven fish contained mild to moderate amounts of g/f.

Cold Water, Final – Seven fish had mild amounts of g/f.

Cold Water, Moribund – seven fish lacked g/f and seven fish contained mild to moderate amounts of g/f.

Cold Water, Swim – Up – all eight fish had mild to moderate amounts of g/f.

Cold Water, WTR – one fish lacked g/f in hepatocytes and three had mild to moderate amounts.

Results, Examination of Muscle Tissue:

Significant muscle pathology was seen in several fish held in cold water, but was especially evident in moribund fish. This was also true in moribund fish held at the Intermediate temperature. Pathological changes consisted of severe degeneration of muscle fibers including focal necrosis of individual fibers and myolysis and/or edema. There also appeared to be attempts to repair muscle fibers (rowing) where hypertrophied nuclei had aligned themselves in an attempt to repair the lesions (figures 6-9).

Warm Water, Final – muscle tissue was mostly normal.

Warm Water, Moribund – muscle tissue was mostly normal. There was mild hypertrophy of muscle fiber nuclei in one fish and mild to moderate rowing of muscle fibers with edema in two fish.

Warm Water, Swim – Up - muscle tissue was mostly normal.

Warm Water, Final - – muscle tissue was mostly normal.

Intermediate Water, Final – There was mild necrosis and associated myophagia (phagocytosis of degenerate muscle) in four fish. In addition, there was mild to moderate rowing in three fish.

Intermediate Water, Moribund - Four moribund fish showed moderate rowing of muscle fibers with edematous change. Also, hypertrophy of muscle fibers in two fish.

Intermediate Water, Swim – Up – muscle tissue of all fish was mostly normal.

Intermediate Water, WTR - – muscle tissue of all fish was mostly normal.

Cold Water, Final – Four fish were normal, but four fish showed severe degeneration with rowing of muscle fibers and edema associated with rowing. Hypertrophy of muscle fibers was apparent in one fish.

Cold Water, Moribund - Five fish showed mild degeneration with rowing of muscle fibers and four showed moderate to severe degeneration with rowing. Edema was associated with rowing. Necrosis of muscle fibers was seen in one fish.

Cold Water, Swim – Up - mild to moderate rowing of muscle fibers was apparent in seven fish.

Cold Water, WTR - muscle tissue of all fish was mostly normal.

Results, Examination of kidney tissue:

With the exception of mild swelling of kidney tubules in a few fish, excretory kidney was mostly normal (figure 1). However, mild degenerative changes were seen in hematopoietic tissue of several fish held in warm, intermediate and cold temperatures. These consisted in degeneration of hematopoietic cells and erythrocytes that had been phagocytized and accumulations of the wear and tear pigment, lipofuscin (figure). These changes were more common in fish held in cold water.

Warm Water, Final – most kidney tubules were normal. There was mild degeneration in hematopoietic tissue and small amounts of lipofuscin in two of eight fish.

Warm Water, Moribund – kidney tubules of four fish were normal. Degeneration was noted in hematopoietic tissue of three fish.

Warm Water, Swim – Up – kidney tubules of most fish were normal, although there was mild swelling in three fish. Mild degenerative changes were present in hematopoietic tissue of most fish.

Warm Water, WTR - kidney tissue, both excretory and hematopoietic, were mostly normal.

Intermediate Water, Final – kidney tissue, both excretory and hematopoietic, were mostly normal.

Intermediate Water, Moribund – kidney tubules were mostly normal, however there was mild degenerative changes in hematopoietic tissue of four fish.

Intermediate Water, Swim – Up - kidney tubules were mostly normal, however there was mild degenerative changes in hematopoietic tissue of four fish.

Intermediate Water, WTR - – kidney tissue, both excretory and hematopoietic, were mostly normal.

Cold Water, Final - kidney tubules were generally normal, however, there was mild swelling of tubules in two fish. There was mild degeneration of hematopoietic tissue of two fish and mild accumulations of lipofuscin pigment in three fish.

Cold Water, Moribund - kidney tubules were generally normal, however, there was mild swelling of tubules in three fish. There was, however, mild degeneration of hematopoietic tissue of five fish and mild accumulations of lipofuscin pigment in six fish.

Cold Water, Swim – Up - kidney tubules were mostly normal, however, there was mild tubule swelling in four fish. Mild, degenerative changes were present in six fish and mild accumulations of lipofuscin in three.

Cold Water, WTR – kidney tubules of all fish were mostly normal, as was hematopoietic tissue.

Results, Examination of Gills:

Warm, Intermediate and Cold Waters: - gills of all fish were mostly normal. While hypertrophy of gill epithelium was seen in an occasional fish, this change was mild in all cases.

Legend to Figures:

Figure 1. A small amount of yolk (Y) is seen in visceral cavity of a moribund fish held in cold water. X250

Figure 2. A moderate amount of yolk (Y) is present in visceral cavity of a swim-up fry held in cold water. Note liver (L) adjacent to yolk. X250

Figure 2.3. Normal adipose tissue (A) present in visceral cavity of a trout held in warm water is surrounded by pancreatic tissue (P). X250

Figure 4. Note atrophied visceral fat in visceral cavity of trout held at an intermediate water temperature. X250

Figure 5. Note lack of vacuolation in hepatocytes of swim-up fry held in cold water. X250.

Figure 6. Shows normal muscle fibers from trout held in warm water. X250.

Figure 7. Necrotic muscle fibers (arrows) are seen surrounded by degenerate muscle fibers attempting to repair themselves as evidenced by the hypertrophied nuclei and significant “Rowing” of nuclei.X250

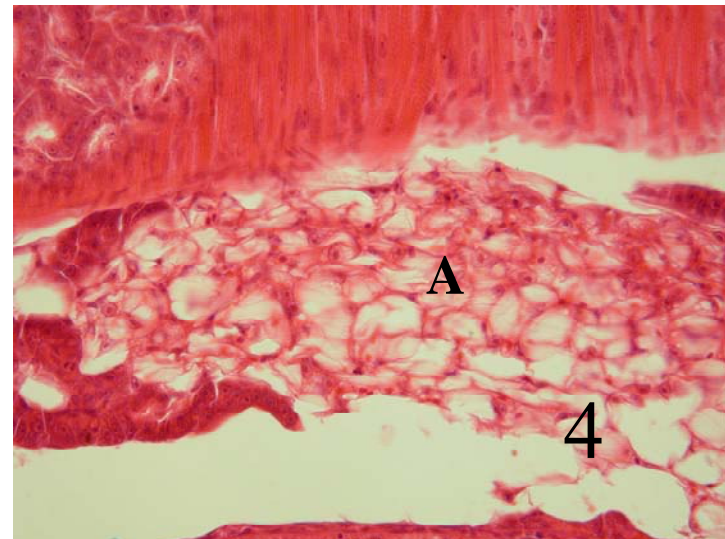
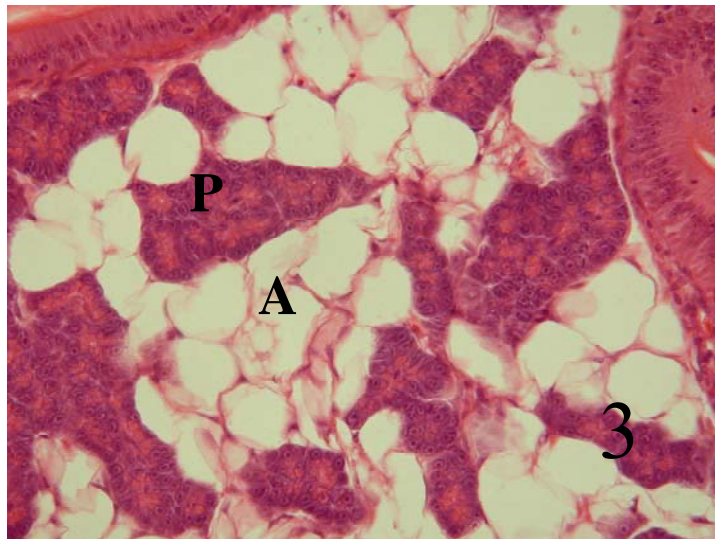
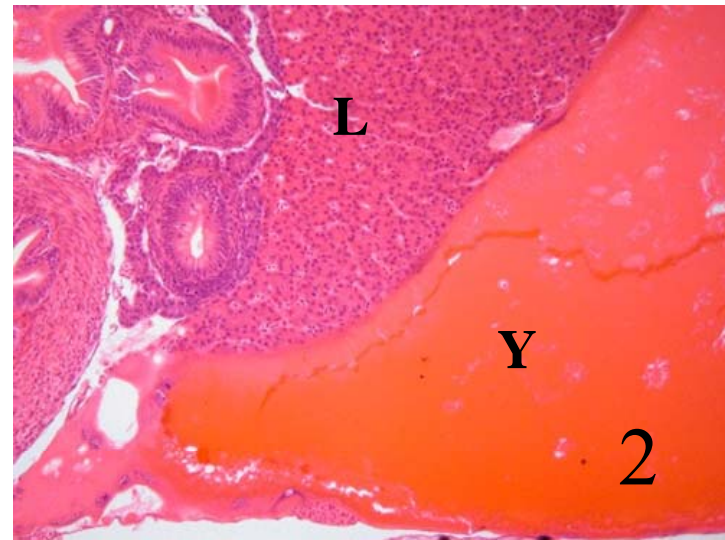
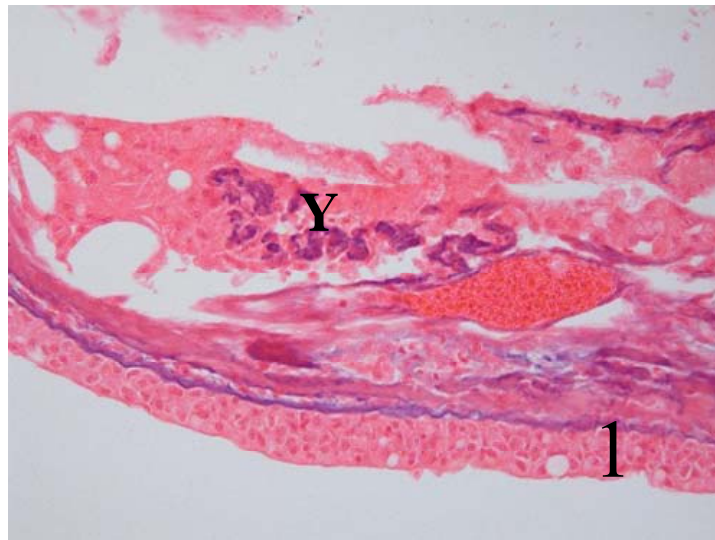
Figure 8. Moribund trout held in cold water. Some muscle fibers show myolysis and/or edema (block arrow). Note repairing of muscle fibers (rowing) where hypertrophied nuclei have aligned themselves in an attempt to repair the lesions (arrows). X250

Figure 9. Degenerate muscle fibers have lost striations. Note hypertrophied nuclei (arrow) and “Rowing” of nuclei (block arrow). Moribund trout held in cold water.X500.

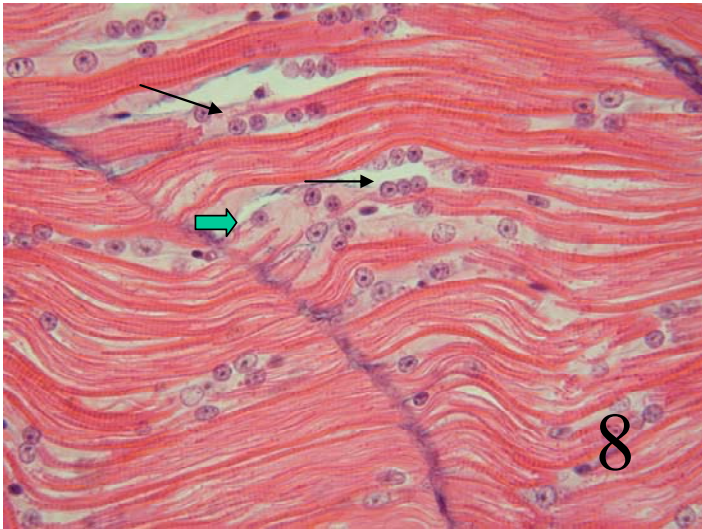
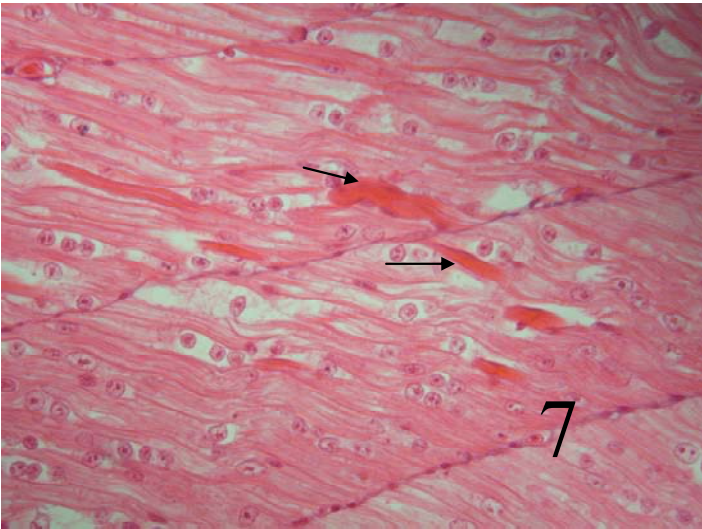
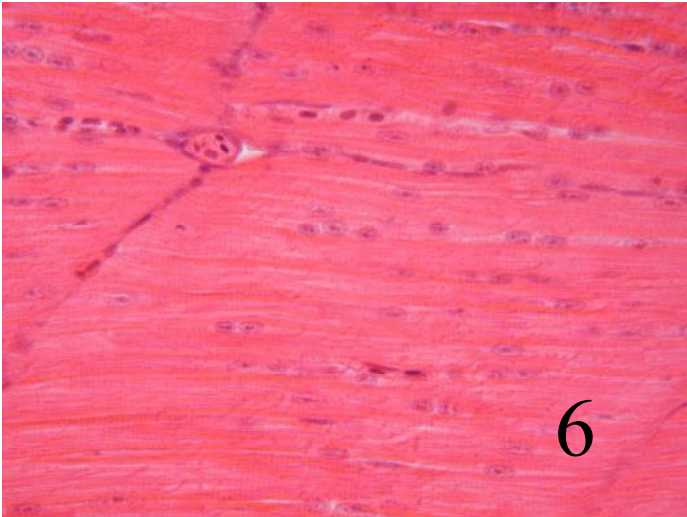
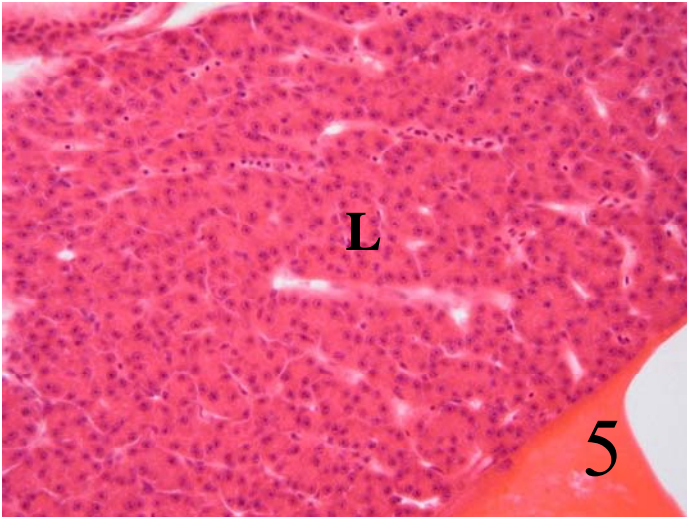
Figure 10. Section of normal kidney from fish in warm water shows hematopoietic tissue (arrow) and kidney tubules (block arrow). X125

Figure 11. Kidney tubule (T) is quite normal, but hematopoietic tissue contains few hematopoietic elements. Macrophages are common (arrows). Moribund fish from intermediate temperature.X500

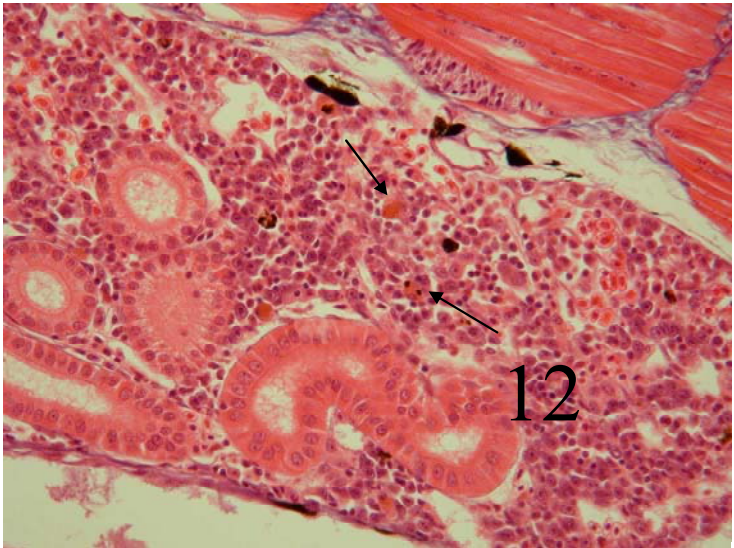
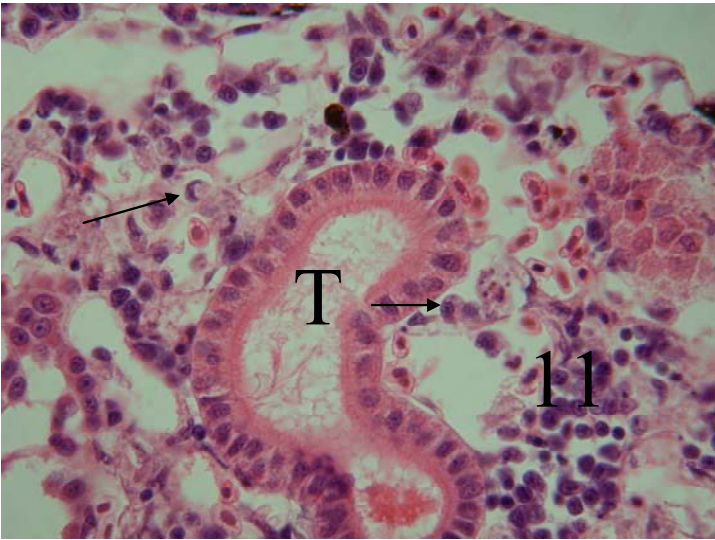
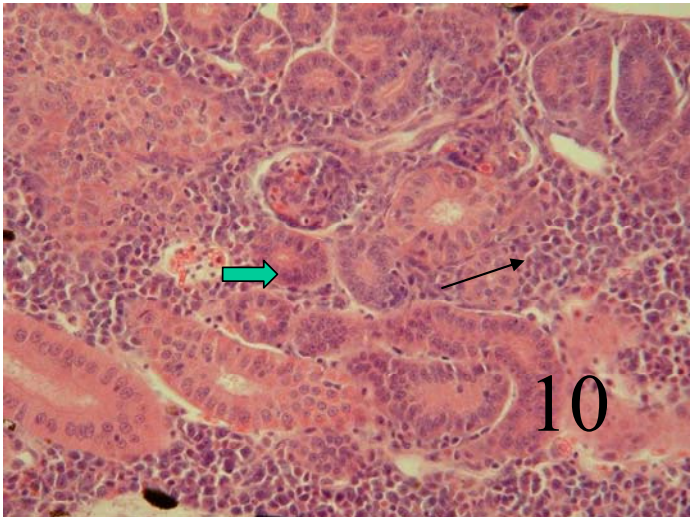
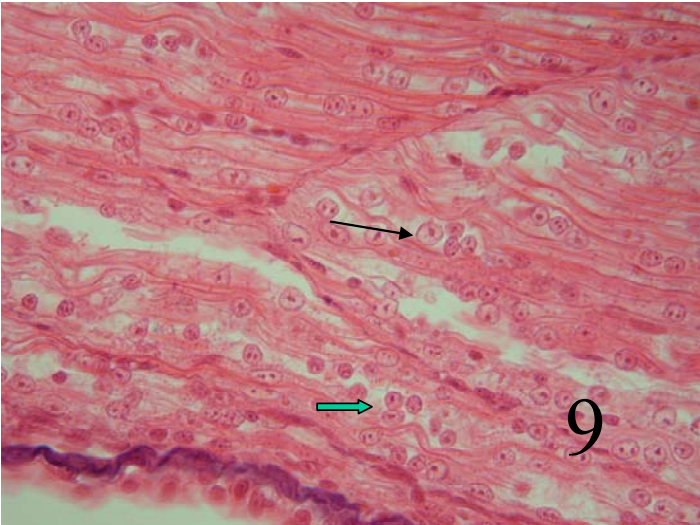
Figure 12. . Degenerate cells in hematopoietic tissue have been phagocytized by macrophages Swim-up, warm temperature (arrows).X250



Figures



Figures



Figures

APPENDIX B. TABLE OF FRY COUNTS FROM FIELD STUDY

Comparison of cutthroat trout fry counts in survey reaches of six study streams by 100-meter increments in 2002, 2003, and 2004, including average number of fry per 100 m (Fry/100m), and estimated fry recruitment to the start of winter (FRI; see Part 3).

Distance (m)	Roaring			Columbine			Cabin			Little Vasquez			West			East Fork Sheep																				
	lower	upper		lower	upper		lower	upper		lower	upper		lower	upper		lower	upper																			
	'02	'03	'04	'02	'03	'04	'02	'03	'04	'02	'03	'04	'02	'03	'04	'02	'03	'04	'02	'03	'04															
100	35	*	6	7	11	33	28	*	*	2	9	10	9	22	2	2	0	0	1	1	0	0	*	1	21	*	4	0	*	0	2	0	2	0	0	0
200	23	*	6	15	5	24	7	1	3	1	0	0	10	0	0	3	0	0	2	0	0	0	1	1	20	*	7	0	*	0	8	1	3	0	0	0
300	14	*	5	22	20	10	8	1	4	5	0	4	22	6	4	7	0	0	0	0	0	0	0	0	15	*	3	0	*	0	3	0	3	0	0	0
400	5	*	19	31	5	27	0	7	35	*	*	*	54	4	5	23	1	3	0	2	0	0	2	0	13	*	3	0	*	0	0	4	9	1	0	0
500	8	*	1	44	*	15	- ^a	- ^a	- ^a	*	*	*	36	4	3	24	0 ^b	4	0	1	0	0	0	0	14	*	6	0	*	0	4	0	3	7	0	0
Fry/100m	17.0	*	7.4	23.8	10.3	21.8	10.8	3.0	14.0	2.7	3.0	4.7	26.2	7.2	2.8	11.8	0.2	1.4	0.6	0.8	0	0	0.75	0.4	16.6	*	4.6	0	*	0	3.4	1.0	4.0	1.6	0	0
FRI (fry per 100m)	13.1	*	4.5	14.1	6.2	12.1	15.2	1.8	7.9	1.5	2.2	1.5	16.9	2.3	1.3	5.9	0.1	0.5	0.3	0.2	0	0.0	0.2	0.1	5.5	*	2.1	0.0	*	0	1.3	0.4	1.1	0.6	0	0

* Not surveyed

^a Survey reaches on Columbine Creek were constrained to 300m downstream and 300m upstream overall, due to a large complex of beaver ponds between the reaches, and unsuitable habitat upstream and downstream. Additional beaver ponds built in the upper 100 m of the lower reach in 2003 prevented surveying fry in this reach.

^b Only 50 of 100 m surveyed.

^c Only fry counts from reaches 100 m reaches that were surveyed during all three years were included in estimate of the FRI.

APPENDIX C. STUDY STREAM MEAN DAILY TEMPERATURES

Mean daily temperatures at each station where thermographs were deployed in the six study streams. The solid lines indicate actual data collected while thermographs were deployed. The dotted lines indicate the periods for which temperatures were predicted (see Part 3).

