

# FISHERY RESEARCH



## LABORATORY STUDIES OF GROWTH AND PHYSIOLOGY OF REDBAND TROUT FROM DESERT AND MONTANE STREAMS

FY2006 Progress Report



Prepared by:

John Cassinelli  
Graduate Research Assistant  
University of Idaho

Christine Moffitt  
USGS, Idaho Cooperative Fish and Wildlife Research Unit  
University of Idaho, Department of Fish and Wildlife Resources

Kevin Meyer, Principal Fisheries Research Manager  
Idaho Department of Fish and Game

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**By**

**John Cassinelli  
Graduate Research Assistant  
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**Christine Moffitt  
USGS, Idaho Cooperative Fish and Wildlife Research Unit  
University of Idaho  
Department of Fish and Wildlife Resources**

**Kevin Meyer  
Idaho Department of Fish and Game  
600 South Walnut Street  
P.O. Box 25  
Boise, ID 83707**

**To**

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## ABSTRACT

Redband trout *Oncorhynchus mykiss gairdneri* in the Columbia River basin occupy high elevation mountain streams with high flows and cool temperatures as well as low elevation desert streams with little flow and warm temperatures. We began a two-year laboratory study to evaluate the physiology and growth of selected wild populations exposed to simulated desert or montane daily water temperature regimes to test whether redband trout from a desert stream have evolved to physiologically handle higher water temperatures better than redband trout from a montane stream. We collected gametes from three wild stocks of redband trout (two desert and one montane), performed single parent crosses from these gametes, and incubated and reared these F1 offspring. At about 930 degree days, stocks were tested under either a simulated desert or montane stream water temperature cycle for 35 d. As a control, we also reared gametes from a hatchery stock. The fish were monitored daily, and samples of fish were collected before, during, and at the end of the 35 d trial to evaluate growth and survival, feed efficiency, plasma cortisol, heat shock proteins, and body proximate analysis. The hatchery fish showed higher growth rates and more efficient feed efficiency in both the desert and montane temperature regimes. In all wild populations, growth and feed efficiency were slightly higher in montane temperature regime. The hatchery stock's growth was higher in the desert temperature regime but feed efficiency was higher in the montane regime. Heat shock protein 70 (HSP 70) expression was higher in fish kept in the desert temperature regime. The muscle tissue from the two desert stocks held in desert temperatures showed elevated HSP 70 levels. This was also the case for liver tissue, but the one montane stock held in desert water conditions also had elevated HSP proteins in the liver tissue.

Authors:

John Cassinelli  
Graduate Research Assistant  
University of Idaho

Christine Moffitt  
Professor of Fisheries and Wildlife and Assistant Leader, ICFWRU  
University of Idaho

Kevin Meyer  
Principal Fisheries Research Manager  
Idaho Department of Fish and Game

## INTRODUCTION

In the Columbia River basin, redband trout *Oncorhynchus mykiss gairdneri* occur east of the Cascade Range to barrier falls on the Pend Oreille, Spokane, Snake, and Kootenai rivers and in the upper Fraser River basin (Behnke 1992). In southern Idaho, redband trout are found in the Snake River drainage below Shoshone Falls. Anadromous redband trout, or steelhead, are present in the Snake River below Hells Canyon Dam while resident redband trout occur throughout the basin below the barrier falls.

The habitat that resident redband trout occupy within the Snake River basin varies from high mountain streams with high flows and cool water temperatures to low desert streams with little flow and very warm water temperatures. In southwest Idaho, desert populations of redband trout are found in the Owyhee, Bruneau, and Jarbidge river drainages; Salmon Falls Creek drainage; and smaller tributaries to the Snake River along the Owyhee range. The majority of these streams are low gradient with high spring runoff and low flows into late summer and early fall. During months of low flow, afternoon temperatures have been recorded as high as 32°C for short periods of time (unpublished data, Idaho Department of Fish and Game).

Further north, montane populations of redband trout are found in the Big Wood, Boise, Payette, and Weiser river drainages. Most streams in this montane habitat are steeper gradient with moderate to high flows. Higher elevation and thicker canopy cover results in cooler water temperatures in these streams.

In 1995, all redband trout in the Snake River upstream of Brownlee Reservoir to Shoshone Falls were petitioned for listing under the Endangered Species Act (ESA). This petition was denied (U.S. Federal Register 1995), as insufficient information was provided to demonstrate that the interior redband trout population of the middle Snake River was a distinct population segment. Currently, redband trout are considered a species of special concern by the U.S. Fish and Wildlife Service and the American Fisheries Society and are classified as a sensitive species by the Idaho Department of Fish and Game, the U.S. Forest Service, and the Bureau of Land Management (Knudson 2002).

Redband trout occupy a wide range of habitat and environmental conditions in southern Idaho. Beiting et al. (2000) report the upper critical temperatures for *Oncorhynchus mykiss* ranged from 26.9 to 29.8°C depending on acclimation temperature. However, Behnke (1992) and Zoellick (1999) have both reported desert populations of redband trout feeding actively at temperatures from 26 to 28°C in the Owyhee and Big Jacks drainages. Behnke (1992) supports the hypothesis that the redband trout of western Idaho have evolved adaptations to live in harsh environments characterized by extremes in water temperature and flow. Dwyer et al. (1981, 1983) reported that hatchery redband trout originally from the Catlow basin, Oregon, had optimum growth efficiency at higher temperatures than typically observed for most other salmonids. Evidence regarding the temperature tolerance for redband trout is limited, mixed, and based largely on unpublished data on a hatchery stock. Because of differences in performance between wild and hatchery fish, Gamperl et al. (2002) urged researchers to use wild trout when evaluating hypotheses concerning the physiology of wild populations.

The objective of this study was to explore whether differences in physiological metrics, growth, and survival were detectable between desert and montane populations in their ability to inhabit warm (desert) or cool (montane) water temperatures. This report summarizes the preliminary results of the first year of a laboratory-based evaluation of these characteristics.

## OBJECTIVES

1. Compare the survival, growth, and physiological status of redband trout from desert and montane streams that were exposed to diurnally fluctuating water temperatures similar to conditions in desert and montane streams of southern Idaho.

## METHODS

### **Collection and rearing of experimental stocks**

Ripe male and female redband trout were collected in March and April from two desert streams (Shoofly and Jump creeks), one montane stream (Keithly Creek), and one hatchery source (Hayspur Hatchery, Idaho Department of Fish and Game) (Figures 1 and 2). These wild stocks were chosen because there were no records that these drainages had been stocked with hatchery rainbow trout. Eggs and milt were collected from live fish at the streams, placed into separate plastic bags, and charged with oxygen. These gametes were transported on ice to the University of Idaho fisheries wet laboratory by commercial airplane, and on arrival, gametes were combined into single parent crosses using a matrix mating process to maximize variation. Fertilized eggs were water hardened in 100 ppm iodophor, and then transferred to Heath incubators and reared to hatching. After hatching, the alvins of each stock were enumerated and transferred to troughs and fed a commercial starter trout diet. Stocks were roughly standardized to the same size and degree-days by manipulating the water temperatures.

### **Design, temperature regimes, and sampling**

To begin the trial, fish were weighed (g) and a portion of each group was measured for total length (mm). Fish from each stock were assigned at random to one of four 125 liter circular tanks for a total of 16 tanks in the study. Each tank with fish from Jump Creek, Keithly Creek, and Hayspur Hatchery contained 150 fish; tanks with fish from the Shoofly Creek stock contained 80 fish because of reduced numbers of fish available. Water levels in the tanks were adjusted to allow for similar densities (fish/L) for each tank. The tanks of fish were then assigned randomly to one of two temperature regimes, for a total of eight tanks per temperature regime, two tanks per stock within each temperature. One temperature regime represented the cooler montane environment with a daily range from 9–16°C. A second regime represented the warmer desert environment with a daily temperature range from 18–26°C (Figure 3). Temperatures were fluctuated daily in planned cycles by controlling the amount of chilled, heated, and ambient water entering mixing head boxes (one for each temperature). These temperature fluctuations mimicked diurnal temperatures observed in desert and montane streams as much as possible (Figure 4). In addition, the laboratory desert temperatures were increased somewhat to test the upper lethal limits. Fish were exposed to these daily cycles continuously for 35 days. Water temperatures were recorded throughout the trial with Hobo data loggers (Onset Computer, Bourne, Massachusetts) at 15 minute intervals.

Fish were fed a predetermined ration of commercial fish feed (BioOregon grower) twice daily to satiation. Uneaten food remaining in the tanks was estimated and feed consumption recorded per day for each tank. These data were used to calculate the feed conversion and feed efficiency for each tank of fish. During the tests, natural photoperiod cycles were provided with fluorescent lights to simulate those of the Moscow latitude.

At the beginning, middle, and end of the study (days 0, 17, and 35), fish were removed from tanks, euthanized with tricaine methosulfate, and muscle and liver tissues were removed, placed in labeled bags and stored at  $-80^{\circ}\text{C}$  until they could be analyzed for heat shock proteins (Werner et al. 2005). At the middle and end of the study, fish were removed, euthanized, and stored at  $-20^{\circ}\text{C}$  for proximate analysis (Selong et al. 2001). At the end of the study, all fish were weighed and measured, and blood was collected from a sample of fish by caudal severance in heparinized hematocrit tubes and centrifuged to separate red blood cells. The plasma was retained and frozen ( $-80^{\circ}\text{C}$ ) for determination of plasma cortisol (Iwama et al. 2004).

Change in weight and length of fish in each tank was calculated for the testing interval, and an average daily change was estimated. We calculated a feed efficiency from the change in wet weight in g /g of feed consumed and feed conversion ratio using the estimated weight of feed consumed divided by the change in body weight.

Heat shock proteins were extracted from liver and muscle samples. Samples of muscle tissue were quantified for individual fish within a tank, but a pooled sample of livers was analyzed from each tank due to small sample quantity. Quantification of proteins was accomplished with western blots; blot membranes were scanned to a digital image, and density of each blot was determined using ImageJ software (Rasband 1997). For each sample, a ratio was calculated by dividing the density of each blot on a gel by the density of a human standard from that same gel.

## RESULTS

Little mortality occurred in any of the test tanks of fish. The Hayspur stock of fish showed rapid growth and highest feed efficiencies (Figures 3, 4, and 5). Among the wild fish stocks, Jump Creek fish had higher daily growth and higher feed efficiency followed by Keithly and Shoofly (Table 1; Figure 5). Hayspur fish grew more than 0.8 mm per day, with the highest growth in the desert temperature regime (Table 1). For all three wild stocks, growth rates were generally higher in the montane regime than the desert regime. Feed efficiency for Hayspur, Jump and Keithly stocks was higher for montane regime, but fish from Shoofly Creek were most inefficient in both water temperatures (Table 1; Figure 7).

Heat shock protein 70 (HSP 70) levels were higher in fish from the desert treatments in both muscle and liver tissues (Figures 8 & 9). Within the muscle, levels of HSP 70 varied more among treatments for the two desert stocks (Figure 8). This was also true for liver tissue, but there was also a large separation of HSP 70 levels between treatments for the Keithly stock (Figure 9).

Blood cortisol did not differ greatly among treatments and groups, and levels for all groups were relatively low ( $<30\text{ng/ml}$  for all tanks).

## DISCUSSION

Hatchery fish had consistently faster growth rates and higher feed efficiencies. These results were not surprising, as these fish have been selected for efficient growth in a contained environment. The behavior of hatchery fish was consistently different from the behavior of the wild fish. The hatchery fish came to the water surface to feed, and wild fish remained near the

bottom of the tank even during feeding. It was somewhat surprising that the Hayspur hatchery fish grew better in the desert temperature regime than the montane regime, as this fish should have been under a higher level of stress.

Sonski (1984) reported maximum growth of redband trout occurred at 20°C and declined at 22.8°C. Wild populations of redband trout generally showed faster growth in the montane temperature regime than in the desert regime, but Shoofly fish showed poor growth regardless of the temperature regime. These results are interesting because we would expect that the desert stocks could be more or equally efficient in the desert regime, and that seems to be the case for Shoofly Creek but not for Jump Creek. However, the Jump Creek fish appear more closely related to the Hayspur fish in growth and feed efficiency. Perhaps the Jump Creek fish have had some hatchery influence although records from the Bureau of Land Management and Idaho Department of Fish and Game do not indicate any past stocking. Jump Creek is a direct tributary to the Snake River but flows rarely reach the confluence because most water is diverted for irrigation. Under spring runoff conditions, fish from the Snake River system could have access to Jump Creek. Future work will be needed to resolve these apparent differences between the two desert stocks.

The cellular stress response protects organisms from damage resulting from exposure to a wide variety of stressors including heat stress (Kllemade and Mothersill 2001). HSP 70 expression is a tool to measure this cellular stress response. Different levels of HSP 70 expression between stocks and treatments may provide a useful tool in separating the results of the two treatments. We anticipate further analysis of these data, and perhaps a combination with other variables from these treatments in a multivariate approach, will assist in the separation of these stocks.

Protein and lipid analysis will provide valuable information of overall body condition of these stocks both in the middle and at the end of our study. Those results were not yet available at the time of this report.

For the second year of this study, we plan to repeat the study with a second montane population added to the design. This was our intention in the first year of the study but snow pack and spring runoff made finding a second pure montane strain of redband a difficult task. We will also discuss whether or not to repeat this study with the same desert populations. Because there was much variation in the responses of our two desert populations, it may be beneficial to incorporate a different desert population as well. The laboratory design of this experiment needs to be further considered due to a lack of power from low numbers of replicates. Our design was limited by numbers of fish and fish size and behavior. Some options for next year include adding more tanks or mixing fish if they are more compatible. After results from this first year of this study are fully analyzed, the study will be appropriately modified and repeated again for a second year to further investigate our initial findings and questions.

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Table 1. Summary of growth (weight and length), feed consumption, feed efficiency, and feed conversion ratio for replicate tanks of redband trout over a 35-day temperature trial with a desert and montane temperature treatment.

<b>Stock and Replicate</b>	<b>Fish Weight (g) Initial</b>	<b>Fish Weight (g) Final</b>	<b>Average Daily Growth (g)</b>	<b>Average Growth (mm)</b>	<b>Daily Feed Eaten (g) Per Fish</b>	<b>Feed Efficiency</b>	<b>Feed Conversion Ratio</b>
<b>Desert</b>							
Jump-1	0.55	2.01	0.041	0.63	0.057	0.71	1.41
Jump-2	0.83	1.92	0.038	0.63	0.057	0.67	1.50
Shoofly-1	0.25	1.11	0.024	0.48	0.057	0.42	2.41
Shoofly-2	0.38	1.01	0.018	0.47	0.056	0.32	3.18
Keithly-1	0.52	1.49	0.027	0.55	0.062	0.43	2.30
Keithly-2	0.49	1.35	0.024	0.51	0.055	0.43	2.33
Hayspur-1	0.57	3.74	0.088	1.09	0.123	0.71	1.40
Hayspur-2	0.57	3.74	0.088	0.92	0.103	0.85	1.17
<b>Montane</b>							
Jump-1	0.51	2.40	0.053	0.75	0.054	0.98	1.02
Jump-2	0.59	2.29	0.047	0.78	0.055	0.86	1.17
Shoofly-1	0.30	1.11	0.023	0.53	0.055	0.41	2.43
Shoofly-2	0.33	1.43	0.031	0.64	0.072	0.43	2.35
Keithly-1	0.80	1.46	0.026	0.57	0.056	0.46	2.16
Keithly-2	0.53	1.71	0.033	0.66	0.049	0.67	1.49
Hayspur-1	0.53	3.52	0.079	0.88	0.079	0.99	1.01
Hayspur-2	0.57	3.36	0.077	0.88	0.079	0.98	1.02

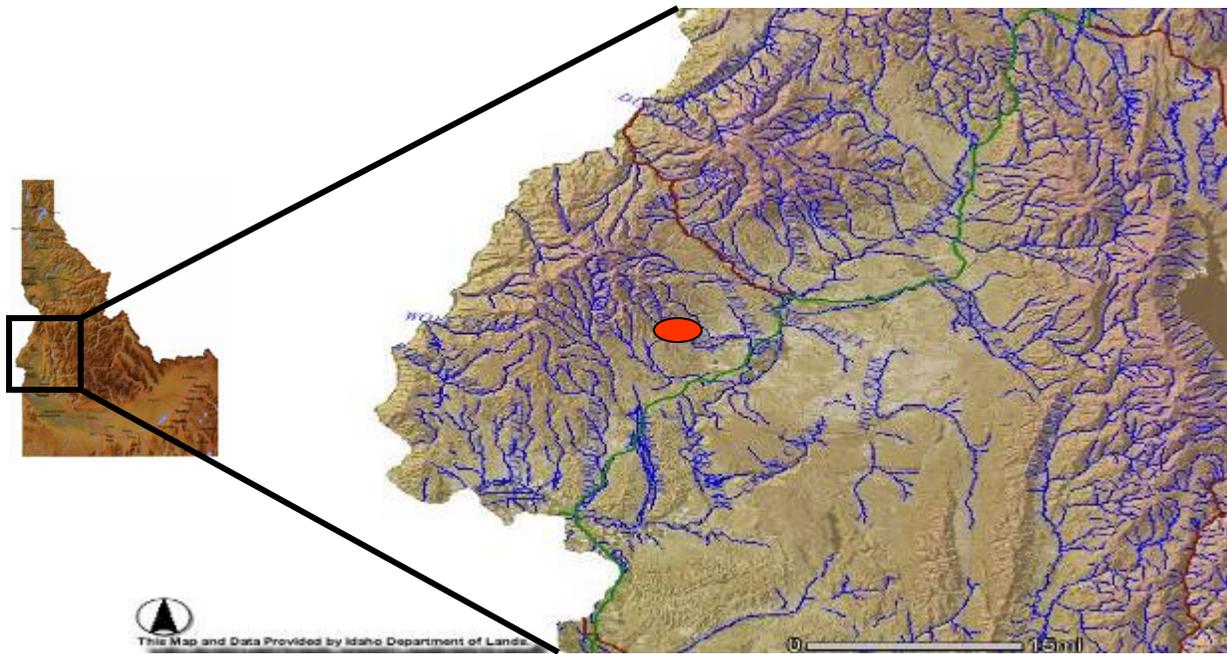


Figure 1. Map of the montane Keithly Creek site. The red dot represents where fish were sampled and gametes collected.

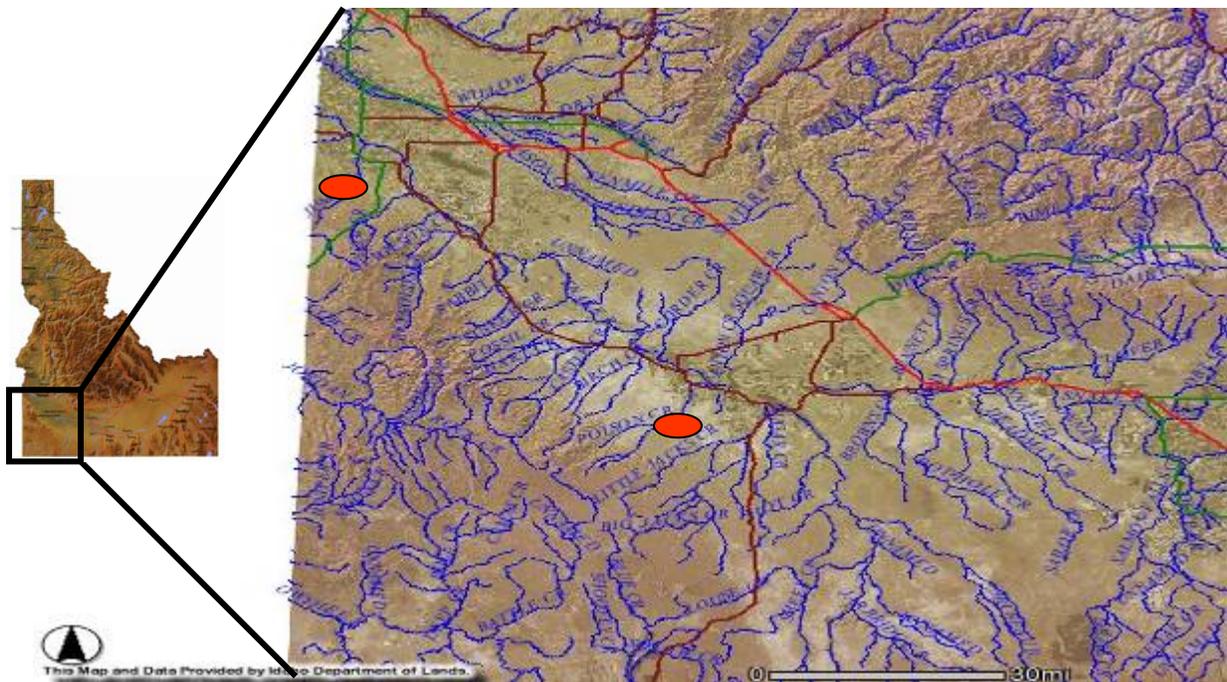


Figure 2. Map of the desert Jump and Shoofly Creek sites. The red dots represent where fish were sampled and gametes collected for each site.

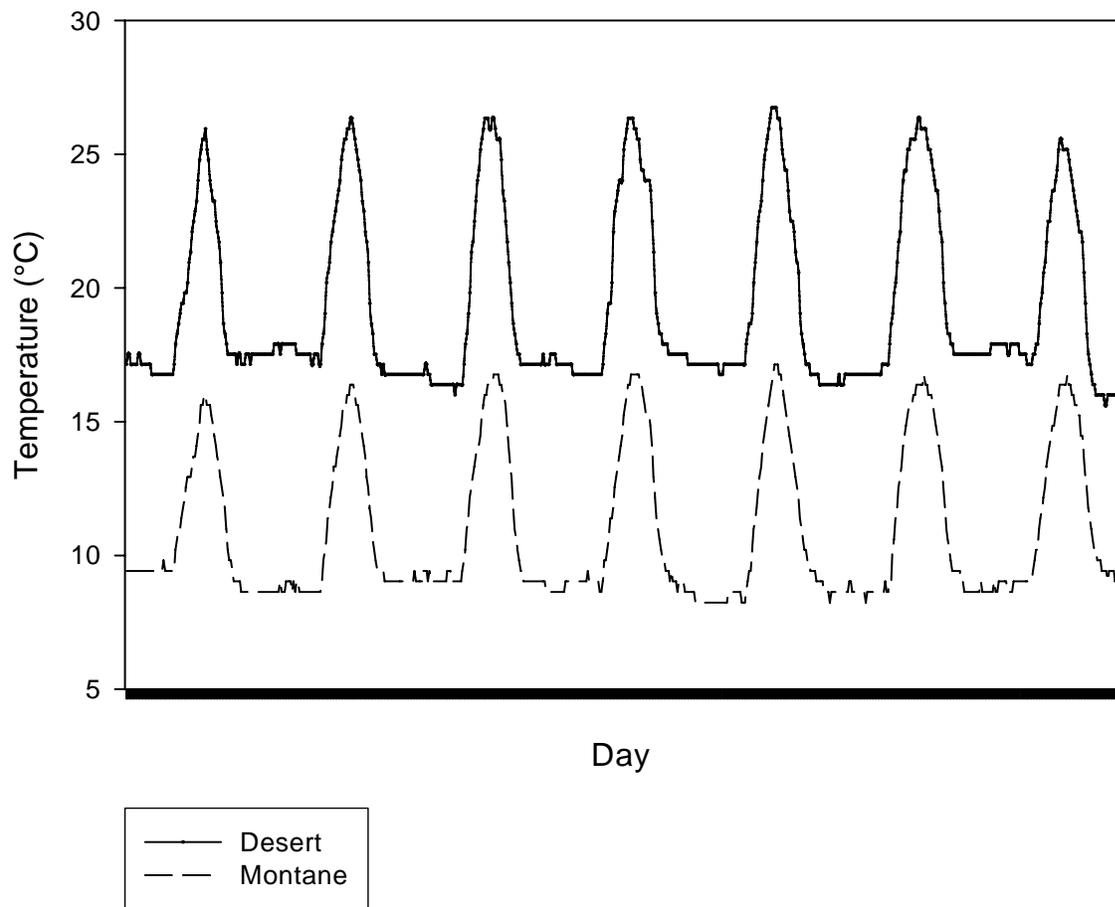


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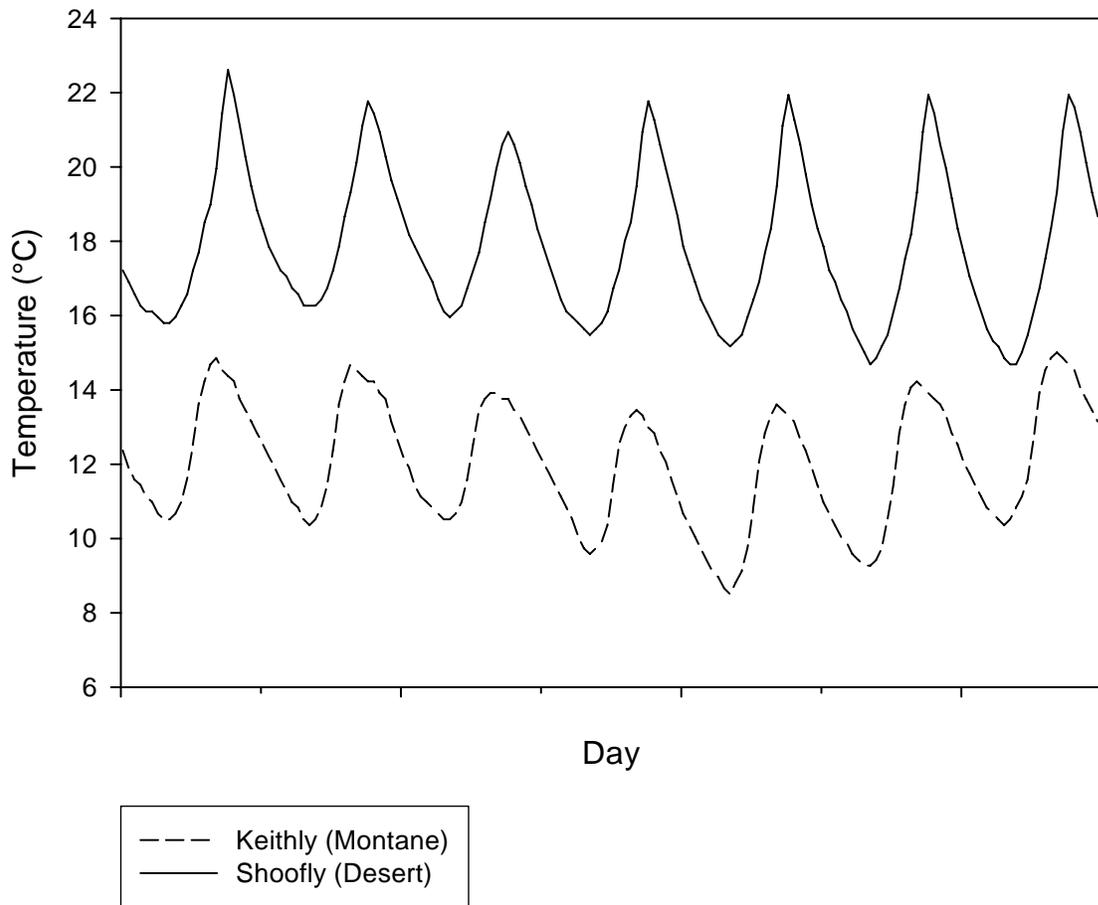


Figure 4. One week (7 days) of daily temperature fluctuations in two wild streams (Keithly and Shoofly) from August 4–10, 2004. Temperatures were recorded every hour on a Hobo thermograph.

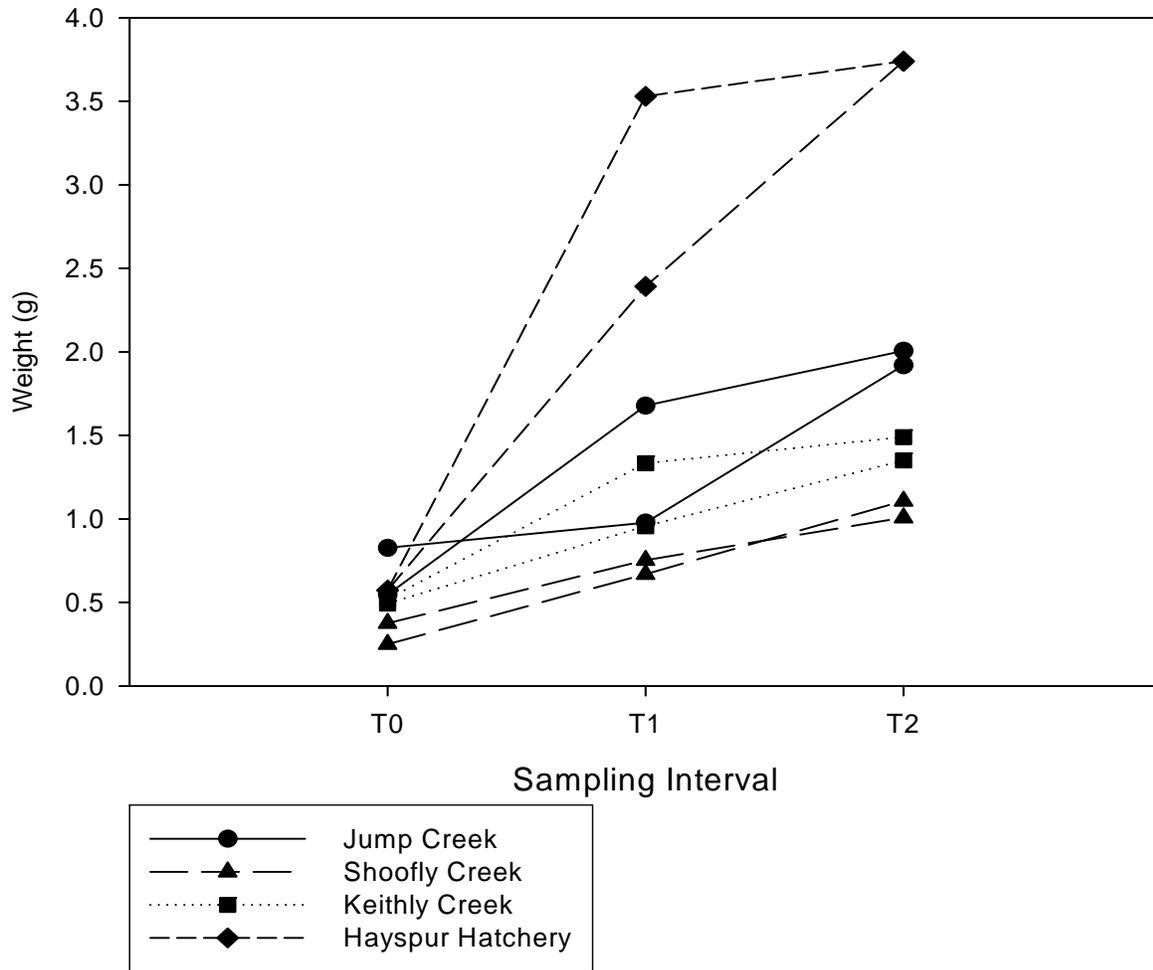


Figure 5. Average daily weight gain (g) of redband trout from three wild stocks and one hatchery stock over a 35-day temperature trial with a desert temperature treatment.

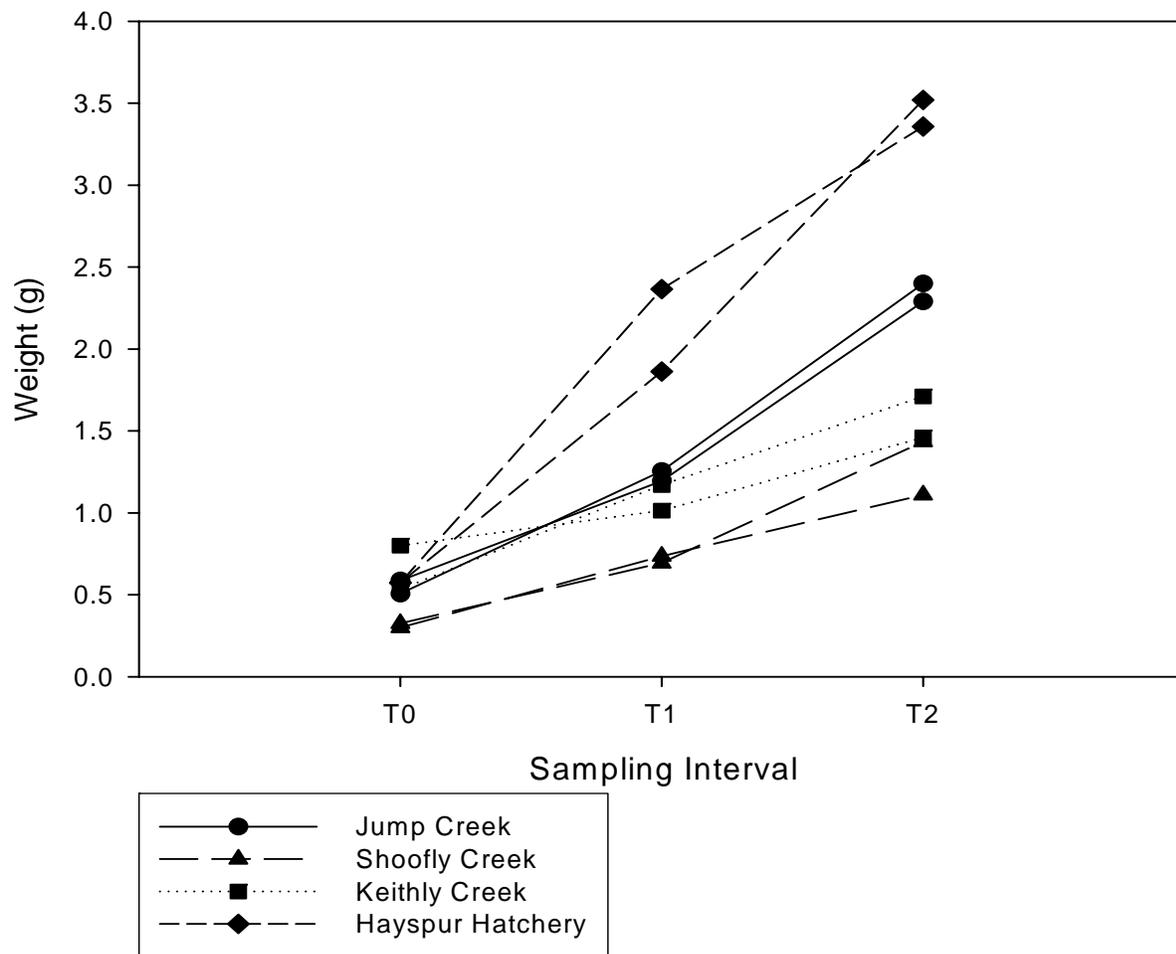


Figure 6. Average weight of fish in each replicate tank by stock at three sampling intervals for the montane temperature treatment. Points for each tank of fish are connected by dashed or solid lines.

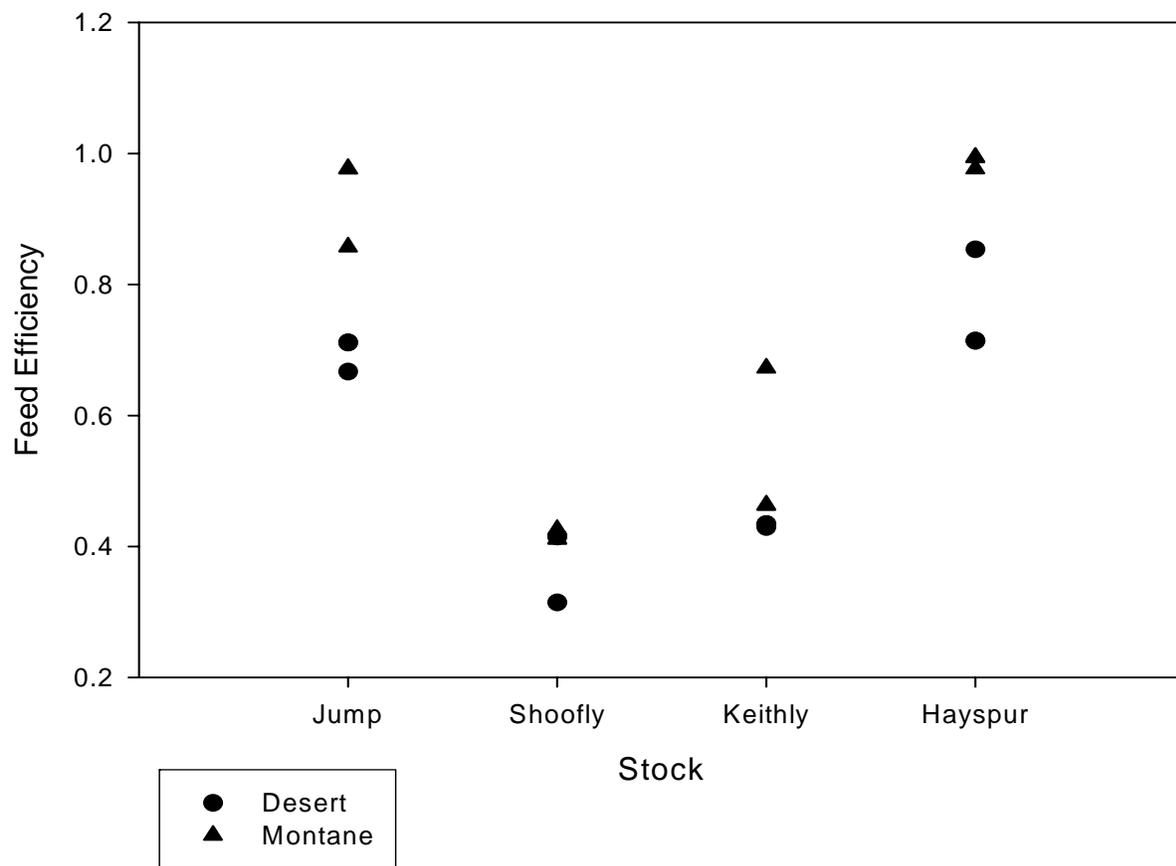


Figure 7. Feed efficiency of redband trout from three wild stocks and one hatchery stock over a 35-day temperature trial with a desert and montane temperature treatment.

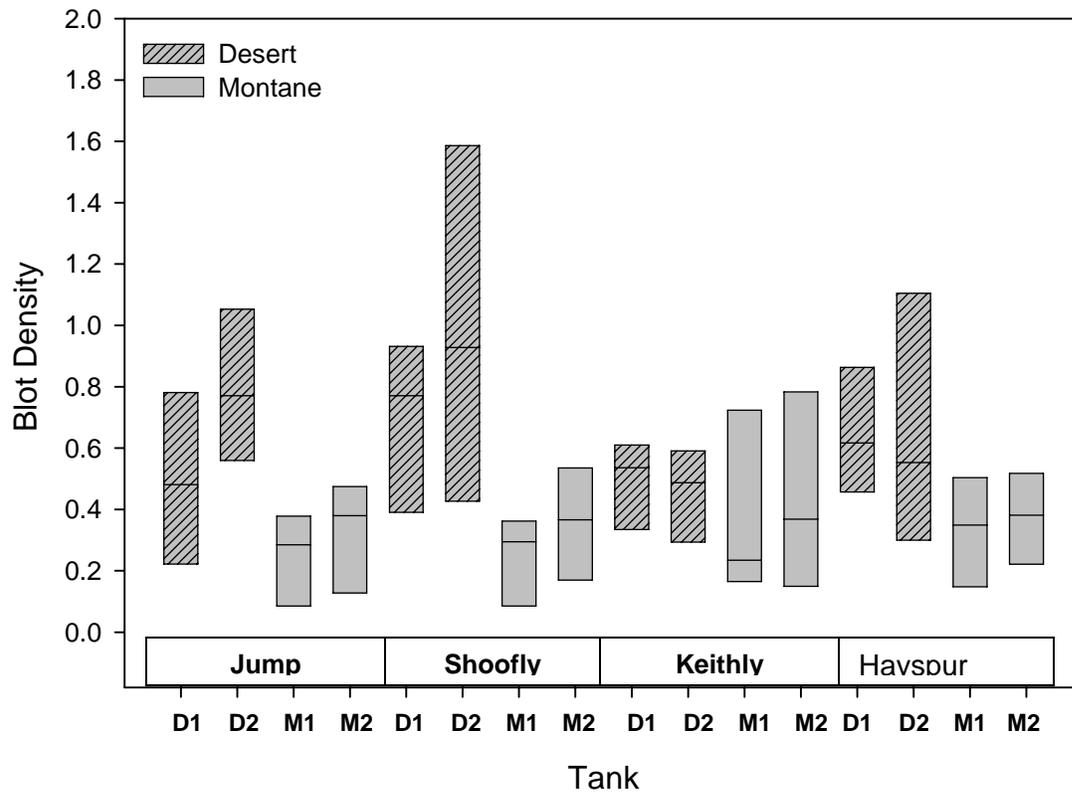


Figure 8. Box plots of heat shock protein 70 relative density for muscle tissue samples from fish at the end of the temperature trial, by desert and montane temperatures. Five fish were sampled from each tanks. Shaded boxes represent desert tanks while gray boxes represent montane tanks.

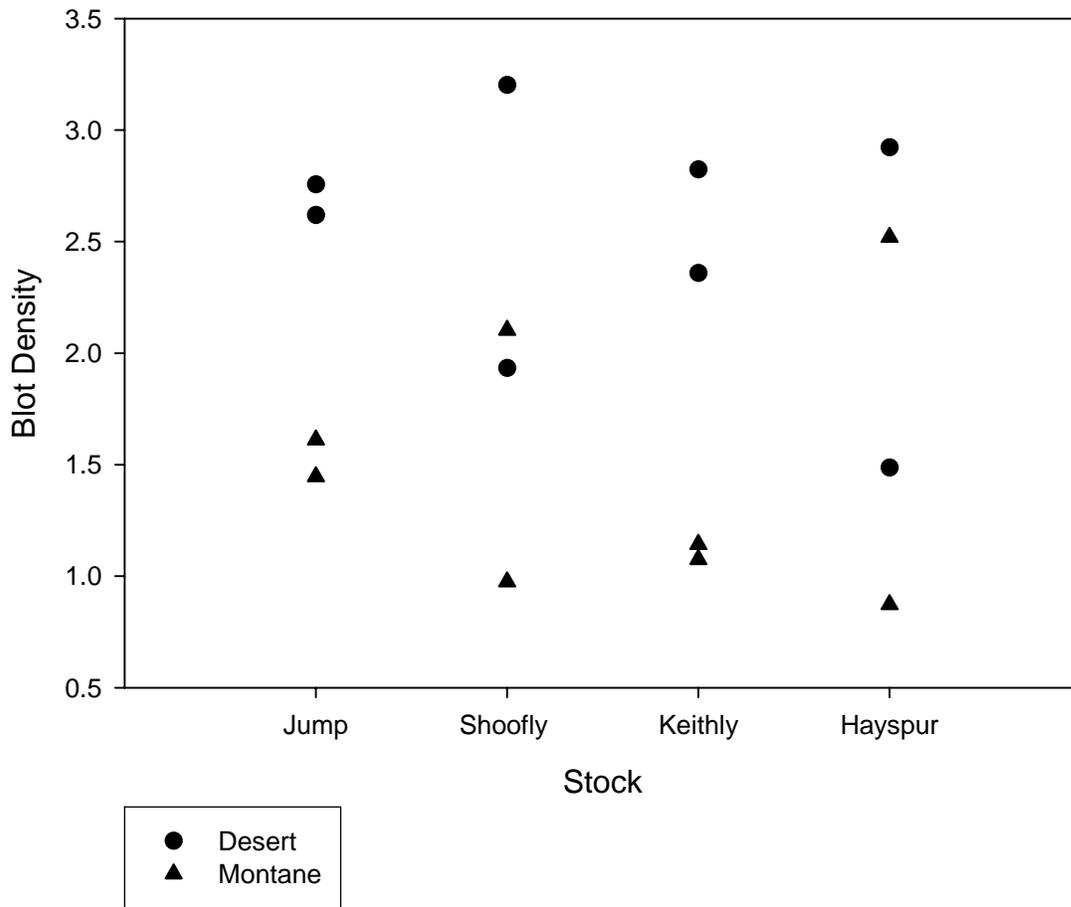


Figure 9. Liver heat shock protein 70 densities by treatment and stock. Five fish were sampled for each tank and the samples were pooled before blotting.

**Prepared by: Approved by:**

IDAHO DEPARTMENT OF FISH AND GAME

John Cassinelli  
Graduate Research Assistant

---

Steve P Yundt, Chief  
Bureau of Fisheries

Christine M. Moffitt  
Professor of Fisheries and Wildlife  
Assistant Leader, ICFWRU

---

Daniel J. Schill  
Fisheries Research Manager

Kevin Meyer  
Principal Fisheries Research Biologist  
Idaho Department of Fish and Game