

**PROJECT TITLE:** Development and Evaluation of Starter Diets and Culture Conditions for 3 Subspecies of Cutthroat Trout and Gila Trout

**REPORT GIVEN IN YEAR** 2010

**PROJECT WORK PERIOD:** 10/1/2005 – 12/31/2009

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**REASON for TERMINATION:** Objectives completed (with exception of those for Gila trout and ongoing outreach) and funds terminated.

**PROJECT OBJECTIVES:** The project had the following objectives:

1. Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout.
2. Determine the effect of water temperature on trout growth, survival, and quality when reared under laboratory conditions.
3. Determine the effect of rearing density on cutthroat trout growth, survival, and quality.
4. Conduct production-scale evaluations of the best diet × temperature × density combinations identified in the first three objectives.
5. Develop outreach products to provide fish culturists and feed manufacturers with information on optimal growth temperatures, optimal rearing densities, and diet formulations for inland cutthroat trout subspecies.

#### **PRINCIPAL ACCOMPLISHMENTS:**

##### **Objective 1 – Effects of diet type on cutthroat trout survival, growth, and quality**

The two principal research institutions, the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC) and Colorado State University (CSU) completed a set of 120-day diet trials on the effects of diet type on the survival, growth, and quality of first-feeding Snake

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<sup>1</sup> Retired from project team in 2007; replaced by Dr. Webb and K. Kappenman.

<sup>2</sup> Retired prior to the production trial; production trial performed by Jeremy Liley of Liley Fisheries.

<sup>3</sup> Left project in 2008 because of ongoing difficulties with rearing Gila trout.

River cutthroat trout (*Oncorhynchus clarki* subsp.; SRCTT), Yellowstone cutthroat trout (*O. c. bouvieri*; YCTT), and Colorado River cutthroat trout (*O. c. pleuriticus*; CRCTT). The YCTT and SRCTT trials were conducted at 10.0°C, while the CRCTT trial was conducted at 10.5°C. A similar study was planned for Gila trout (*O. gilae*) at the U.S. Fish and Wildlife Service Mora National Fish Hatchery and Technology Center, but repeated production problems over the 2 years that the location received funding prevented the collection of sufficient data at that location.

The diets used in the study were the following: Rangen Regular Trout (RRT), Rangen Soft Moist (RSM), Silver Cup Regular Trout (SCRT), Silver Cup Soft Moist (SCSM), Skretting Nutra-Plus (now Bio-Vita Fry; SNP), Skretting Nutra-Plus w/21 days of *Artemia* supplementation (CSU only; SNP+A), Experimental 601 (E601), and Experimental 602 (E602).

These diets were selected for inclusion in the study because they were currently available commercial diets or were open-formula formulations that could be easily produced by fish feed manufacturers. It was not deemed efficient to try to produce a cutthroat trout specific diet because of the relatively small size of the potential market. All diets were fed at 4% wt./d, which turned out to be an *ad libitum* ration.

The best diet in terms of both survival and growth for SRCTT was SNP, with fish gaining an average of 4.78 g/fish over the 120-d trial and survival exceeding 97%. The best diet for YCTT was also SNP, with fish fed that diet gaining 3.73 g/fish and survival of > 97%. With the CRCTT, the best non-supplemented diet in terms of growth (3.89 g/fish) and survival (77.5%) was also SNP. However, when the SNP+A diet is included, the *Artemia* supplementation led to a 31% increase in final fish size (5.12 g/fish) and a 5.7% increase in survival. Dorsal fin index, a measure of fin condition used as a metric of fish performance, was unaffected by diet for all three cutthroat trout subspecies tested.

One interesting side-note from the CRCTT studies was that the different diets produced significant differences in fish color. When subjected to a quantitative ranking by a team of trained observers, E601 produced fish with the deepest reds, followed by SNP, SNP+A, RRT and SCSM, SCRT, and, at the yellow end of the spectrum, RSM and E602.

An additional 65-d diet trial was performed at the BFTC to compare the effects of different diet supplements on the growth, survival, and performance of SRCTT. The supplements used were the following: Skretting/Bio-Oregon Bio-Vita (SNP), Skretting/Bio-Oregon Bio-Vita + dry *Artemia* flake (SNP+DA), Skretting/Bio-Oregon Bio-Vita + freeze dried cyclopeeze (SNP+C), Silver Cup Fry Micro Pellet Salmon (SCFMP), Otohime (OTH), Otohime + freeze dried cyclopeeze (OTH+C), and Silver Cup Regular Trout (SCRT).

The results of this additional diet trial demonstrated that other started diets (both premium and non-premium) produced the same high levels of survival (> 93%) with SRCTT as did SNP. Additionally, the difference in weight between the fish fed SNP and those fed SNP+A, SNP+DA, SNP+C, OTH, or OTH+C were not significant, indicating that, for SRCTT at least, there is no clear benefit to diet supplementation, and, more importantly, that the use of any typical premium diet provides a similar level of growth performance.

Based on these results, it is clear that the SNP diet, or a similar high-performance diet, is ideal for first-feeding of juvenile cutthroat trout, irrespective of subspecies. Additionally, supplementation of the prepared diets with live *Artemia* during the first 21 days of feeding can confer a significant growth and survival advantage, particularly in cases where overall survival is lower than that seen at BFTC. Finally, if fish color is important, SNP, SNP+A, or any diet with astaxanthin supplementation (e.g., E601) can dramatically change the appearance of the fish.

## **Objective 2 – Effects of water temperature on cutthroat trout survival, growth, and performance**

Snake River and Yellowstone cutthroat trout were reared at 10, 12, 14, 16, 18, and 20°C in a 120-d growth study on first-feeding fish that were fed with *ad lib* rations (4% wt./d) of SNP. Colorado River cutthroat trout were reared for 120 days at 10, 12.5, 15, 17.5, and 20°C in a study that compared both the effects of temperature and diet (SNP+A) and Rangen Regular Trout, supplemented with live *Artemia* for 21 days (RRT+A). A system failure at CSU forced the termination of the 10°C treatment on CRCTT, but otherwise the study proceeded without complications.

The results from the SRCTT study demonstrated the classic temperature × growth relationship, with increasing growth rates as temperature increased from 10 to 16°C, and a decrease in growth rate between 16 and 20°C. Regression analysis of the growth data identified the optimal temperature for growth as 14.5°C. A similar result was seen for YCTT, with the optimal temperature for growth identified as 14.7°C. Survival of YCTT and SRCTT was unaffected by water temperature, likely because of the high quality rearing conditions at the BFTC. The results for CRCTT also followed the same pattern, irrespective of diet type, with optimal growth temperatures of 15.3°C for fish fed RRT+A and 16.4°C for fish fed SNP+A. A significant difference in actual and predicted growth was noted between diets, with fish fed SNP+A consistently growing larger than their counterparts fed RRT+A at the same temperature. The maximum predicted difference between the two diets at the optimal temperatures was close to 57%. Unlike the SRCTT and YCTT, however, rearing temperature did affect survival in an inverse manner, with generally higher survival at temperatures below 15.0°C, independent of diet. Fin condition was unaffected by temperature for all three subspecies.

The objective 2 studies were able to successfully identify the optimal growth temperatures for rearing Snake River, Yellowstone, and Colorado River cutthroat trout. They also provided information that may prove useful to resource managers trying to understand the bioenergetic responses of cutthroat trout populations in natural or hatchery settings at different temperatures.

## **Objective 3 – Effects of density on cutthroat trout survival, growth, and performance.**

Snake River and Yellowstone cutthroat trout were reared at densities of 50 to 350 fish per tank (100 L tanks) to measure the effects of rearing density on survival, growth, and performance. A similar study was conducted with Colorado River cutthroat trout, but because of the results from the SRCTT and YCTT, the densities were increased to 150, 300, 400, and 600 fish/tank. Fish were reared at the optimal temperatures identified in objective 2 (14.5°C for YCTT and SRCTT; 16.4°C for CRCTT) and were fed *ad lib* rations of SNP.

Growth rates of SRCTT and YCTT were slightly, but significantly affected by rearing density, with fish reared at the 50 fish/tank weighing significantly (20% for SRCTT; 15% for YCTT) than those reared at 350 fish/tank. No differences in survival were observed between YCTT density treatments, but SRCTT reared at the lowest density had slightly higher survival than those reared at 350 fish/tank (97.5% vs. 91.7%). Colorado River cutthroat trout also showed a significant density effect, with significantly lower final wet weights for fish reared at 600 fish/tank (8.1 g/fish) compare to those in the 150, 300, or 450 fish/tank treatments (final wet weights of 11.9, 12.1, and 10.4 g/fish, respectively). Interestingly, despite the significant effects of density on the growth of the three cutthroat trout subspecies, fin condition showed no density effect.

## **Objective 4 – Production-scale trial using Snake River cutthroat trout**

A production-scale growth trial using Snake River cutthroat trout was conducted at the Liley Fisheries facility (formerly the Cline Trout Farms Boulder Facility). First-feeding Snake River cutthroat trout were held at ambient well water temperatures (13 – 14°C during the course of the

study) and were fed SNP at a maximum of 4% wt./d. The goal of this experiment was to see how closely the actual growth of the fish under production conditions matched the predicted growth of SRCTT based on the results previously found in this project.

The results of the production trial showed that the actual final wet weight of the fish (3.62 g/fish) were very close to the predicted wet weights (4.0 – 4.13 g/fish) for fish reared at 13 – 14°C. The slight difference in performance may result from the differences in culture systems, but overall the results showed that it is possible to closely approximate production-level results from the data collected under laboratory conditions.

### **Objective 5 – Develop outreach products on cutthroat trout production**

The primary outreach product for this project, a WRAC Extension publication on cutthroat trout production techniques, is currently being developed. During the course of the project, project personnel delivered a number of presentations to industry meetings (e.g., U.S. Trout Farmers Association, U.S. Aquaculture Association, Colorado Aquaculture Association) and to local, regional, and national fisheries meetings. A project website was developed early on, and portions of it have recently been included in the general WRAC website.

### **IMPACTS:**

#### **Development of culture conditions for first-feeding cutthroat trout**

**Relevance:** In 2004, the aquaculture industry in the Western United States requested a comprehensive research project on the development of culture conditions (diet, temperature, rearing density) for first-feeding cutthroat trout of various subspecies. This request was made because past attempts to culture cutthroat trout using diets and techniques developed for rainbow trout had not been very successful.

**Response:** From 2005 through 2009, a multi-institution team conducted a series of experiments on the effects of diet type, water temperature, and rearing density on the survival, growth, and performance of first-feeding Snake River, Colorado River, and Yellowstone cutthroat trout. An additional production-scale experiment was conducted to verify that results from laboratory studies could be adequately transferred to production-level efforts.

**Results:** The study demonstrated that first-feeding cutthroat trout have the highest growth rates and survival when fed premium trout or salmon diets like Skretting Nutra Plus/Bio-Oregon Bio-vita Fry. Additional benefits in early survival and growth may be gained by supplementing prepared diets with live *Artemia* for Colorado R. cutthroat trout. The optimal temperatures for rearing cutthroat trout are subspecies-specific, ranging from 14.5°C and 14.7°C for Snake River and Yellowstone cutthroat trout, to 16.4°C for Colorado River cutthroat trout. Rearing density does have a slight but significant effect on growth, with fish reared at lower densities growing faster than those reared at higher densities. The production-scale trial demonstrated that the results of the laboratory studies could be used to predict growth rates at a production-scale.

**Impact:** The Colorado Division of Wildlife's Glenwood Springs Fish Hatchery switched to the premium diet used in the Colorado R. cutthroat trout study (like Skretting Nutra Plus/Bio-Oregon Bio-vita Fry) as a direct result of this study. The use of live *Artemia* supplementation during the first 21-d of production has not yet been adopted on a wide scale because of the added cost. However, a state hatchery in Alaska did express interest in using the technique to try to improve early survival of first-feeding grayling.

**Collaborators:** Colorado State University faculty in the College of Natural Resources, University of Idaho faculty in the Cooperative Extension Service, U.S. Fish and Wildlife Service personnel at the Bozeman Fish Technology Center, industry personnel at the Cline Trout Farms, Liley

Fisheries, and Nelson and Sons, Inc..

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**RECOMMENDED FOLLOW-UP ACTIVITIES:**

Additional studies on the performance of these subspecies of cutthroat trout are probably not warranted. However, additional studies on the performance of additional cutthroat trout subspecies, particularly in regards to optimal rearing temperatures, would improve the ability of producers to predict growth rates and yields. Additionally, the western aquaculture industry should continue to work with state and federal agencies to gain greater access to cutthroat trout for production purposes.

**SUPPORT:** Use the format shown below to indicate all sources of funding and additional other support, federal and non-federal, for this project. Specify the name of the “other” sources as a footnote to the table.

YEAR	WRAC- USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other <sup>4</sup>	Total	
2006	\$99,991	\$5,000	\$5,000	\$81,500	\$5,500	\$97,000	\$196,991
2007	\$95,677	\$25,829	\$5,000	\$90,000	\$5,500	\$126,329	\$222,006
2008	\$91,973	\$5,000	\$5,000	\$45,000	\$0	\$77,000	\$168,973
2009	\$35,328	\$8,882	\$20,000	\$0	\$1000	\$29,882	\$65,210
<b>TOTAL</b>	<b>\$322,969</b>	<b>\$44,711</b>	<b>\$35,000</b>	<b>\$216,500</b>	<b>\$12,000</b>	<b>\$330,211</b>	<b>\$653,180</b>

**PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:** Attach a separate list of all publications to date for this project. Under the subheadings of *Publications in print* and *Manuscripts*, list journal articles, popular articles, outreach materials, videos, technical reports, theses, dissertations, etc. (reference the “Transactions of the American Fisheries Society” for the preferred format). Under *Papers presented*, include the author(s), title, conference/ workshop, and date(s).

**SUBMITTED BY:** \_\_\_\_\_ 9/14/2010  
 Title: (Work Group Chair or PI) \_\_\_\_\_ Date

**APPROVED:** Please see attached sheet for TA signature \_\_\_\_\_  
 Technical Advisor \_\_\_\_\_ Date

<sup>4</sup> The “Other” category includes in-kind services provided by: 1) the Montana Department of Fish, Wildlife and Parks including the rearing, incubating, and delivering Yellowstone cutthroat eggs to BFTC; 2) the Colorado Division of Wildlife for obtaining and incubating CRCTT eggs, and for the provision of tanks used at CSU, and; 3) the Jackson National Fish Hatchery for provision of SRCTT eggs throughout the study.

### *Publications in Print*

- Brandt, M. M. 2009. Optimal starter diets and culture conditions for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). Colorado State University, Fort Collins, CO.
- Kindschi, G. A., and coauthors. 2009. Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture* 71(4):325-329.
- Brandt, M. M. 2008. Optimal culture conditions for first-feeding Colorado River cutthroat trout. *The Fishline* 20(2):1-16.

### *Papers Presented*

- Myrick, C. A., M. Brandt, G. Kindschi and F. Barrows. 2009. Getting ahead in a cutthroat world: optimizing culture conditions for Snake River, Colorado River, and Yellowstone cutthroat trout. 60<sup>th</sup> Northwest Fish Culture Conference, Redding, CA.
- M. M. Brandt and C. A. Myrick. 2009. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Western Division of the American Fisheries Society, Albuquerque, NM.
- Myrick, C. A. 2009. Fish nutrition research at Colorado State University. Department of Animal Science Seminar, Fort Collins, CO.
- M. M. Brandt and C. A. Myrick. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society, Loveland, CO
- M. M. Brandt and C. A. Myrick. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Aquaculture America 2009, Seattle, WA.
- Brandt, M. M. and C. A. Myrick. 2008. Getting ahead in a cutthroat world: optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Idaho Aquaculture Association 2008 Annual Meeting, Twin Falls, ID, June 21-22.
- Brandt, M. M. and C. A. Myrick. 2008. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Colorado-Wyoming Chapter American Fisheries Society Meeting, Fort Collins, CO, March 3-6.
- Brandt, M. M. and C. A. Myrick. 2008. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Aquaculture America and World Aquaculture Society Joint Meeting, Orlando, FL, February 9-12.
- Brandt, M. M. and C. A. Myrick. 2008. Determination of optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Colorado Aquaculture Association 2008 Annual Meeting, Mt. Princeton, CO, January 18-19.
- Brandt, M. M. and C. A. Myrick. 2007. Getting ahead in a cutthroat world – performance of Colorado River cutthroat trout fed eight starter diets. 137<sup>th</sup> Annual Meeting of the American Fisheries Society, San Francisco, CA, September 2-6.
- Brandt, M. M. and C. A. Myrick. 2007. Getting a head start: growth of Colorado River cutthroat trout fed eight starter diets. Colorado-Wyoming Chapter American Fisheries Society Meeting, Fort Collins, CO, February 26-March 1.
- Brandt, M. M. and C. A. Myrick. 2007. Getting a head start: growth of Colorado River cutthroat trout fed eight diets. Colorado Aquaculture Association 2007 Annual Meeting, Mt. Princeton, CO, January 19-20.

- Fornshell, G. 2006. Aquaculture in the West. A WRAC Perspective (and other stuff too).  
Colorado Aquaculture Association 2006 Annual Meeting, Mt. Princeton, CO, March 3-4.
- Myrick, C. A. 2004. Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout: an introduction to the upcoming WRAC project. Colorado Aquaculture Association 2004 Annual Meeting, Mt. Princeton, CO, December 10-11.

**PROJECT TITLE:** Development and Evaluation of Starter Diets and Culture Conditions for 3 Subspecies of Cutthroat Trout and Gila Trout

**REPORT GIVEN IN YEAR** 2010

**PROJECT WORK PERIOD:** 10/1/2005 – 12/31/2009

**AUTHOR:** Christopher A. Myrick

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 Kevin Kappenman\* – U.S. Fish and Wildlife Service, Bozeman Fish Technology Center

**REASON for TERMINATION:** Objectives completed (with exception of those for Gila trout and ongoing outreach) and funds terminated.

**PROJECT OBJECTIVES:** The project had the following objectives:

6. Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout.
7. Determine the effect of water temperature on trout growth, survival, and quality when reared under laboratory conditions.
8. Determine the effect of rearing density on cutthroat trout growth, survival, and quality.
9. Conduct production-scale evaluations of the best diet × temperature × density combinations identified in the first three objectives.

Develop outreach products to provide fish culturists and feed manufacturers with information on optimal growth temperatures, optimal rearing densities, and diet formulations for inland cutthroat trout subspecies.

## TECHNICAL SUMMARY AND ANALYSIS:

### Introduction

The western United States was home to a variety of cutthroat trout (*Oncorhynchus clarkii* subsp.) including at least 11 distinct subspecies or strains (Figure 1). These fish, while closely related to the coastal rainbow trout (*O. mykiss*), were primarily distributed in inland drainages of the western states (Behnke 1992). These inland cutthroat trout were able to take advantage of

<sup>5</sup> Retired from project team in 2007; replaced by Dr. Webb and K. Kappenman.

<sup>6</sup> Retired prior to the production trial; production trial performed by Jeremy Liley of Liley Fisheries.

<sup>7</sup> Left project in 2008 because of ongoing difficulties with rearing Gila trout.



a diverse range of habitats, from small headwater streams to large rivers and even very large lakes (e.g., Lake Tahoe). Unfortunately, cutthroat trout numbers have declined precipitously throughout much of their range because of a number of factors, including competition from non-native salmonids (Schade and Bonar 2005), habitat alteration, water diversion and development (Harig et al. 2000), and over-exploitation. Thus, the majority of the subspecies are now found in much reduced ranges, and some, including the Yellowstone cutthroat trout (*O. c. bouvieri*) and Colorado River cutthroat trout (*O. c. pleuriticus*) are reared in state and federal hatcheries for conservation and restoration purposes. One subspecies, the Snake River cutthroat trout, has been more widely used by the aquaculture industry either directly or to create rainbow trout × cutthroat trout hybrids (cuttbows); this subspecies is also reared in significant numbers for put-and-grow stocking.

Fish culturists in the western United States have observed poor survival, growth, and quality of cutthroat trout (*Oncorhynchus clarkii* subsp.) relative to rainbow trout (*O. mykiss*). Unlike rainbow trout, which are produced in large numbers for both direct consumption and for put-and-grow fisheries, the inland cutthroat trout subspecies are primarily produced for put-and-grow species, thus placing a premium on high rates of fry and juvenile survival, and good fry quality (e.g., intact fins). Until recently, however, the rearing of cutthroat trout using techniques, diets, and protocols developed for rainbow trout has been less successful than anticipated. Because of the difference in performance between rainbow trout and cutthroat trout culture, the western aquaculture industry requested assistance in the development of starter feeds and early rearing techniques for inland cutthroat trout subspecies.

The goal of this project was to address this request for more information on cutthroat trout culture techniques by conducting a multi-institutional study on the culture of first-feeding Snake River, Colorado River, and Yellowstone cutthroat trout. The rationale behind using multiple subspecies of cutthroat trout was to allow qualitative inter-specific comparisons. The initial proposal also included the related Gila trout (*O. gilae*) as a study species, but because of repeated failures to procure enough swim-up Gila trout fry, this portion of the study was eventually dropped. The project had the following specific objectives.

1. Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout. Providing a feed that is nutritionally complete is obviously necessary for the intensive culture of fish, but nutritional requirements for less-commonly raised species, such as cutthroat trout, are poorly known. Recent studies on rainbow trout demonstrated that standard fish meal-based feeds may not supply the necessary nutrients to prevent fin erosion (Barrows and Lellis 1999). Additionally, the quality of a diet, particularly the protein and lipid profiles, can have marked effects on fish survival, growth, and performance (Nordgarden et al. 2002). The diets selected (see below) were either currently available commercial diets, or open-formula diets developed by the USDA.
2. Determine the effect of water temperature on trout growth, survival, and quality when reared under laboratory conditions. Growth rates and survival of juvenile salmonids are highly temperature-dependent (Myrick and Cech 2000; Myrick and Cech 2003). Fish growth rates typically increase with temperature to some maximum (optimal growth temperature) before declining as temperatures are increased past that point. Rearing fish at or near the optimal growth temperature generally results in more efficient production.
3. Determine the effect of rearing density on cutthroat trout growth, survival, and quality. Fry rearing density is widely accepted as an important factor in determining the growth rate of fish in hatchery settings, particularly when determining the economic return or cost-benefit ratio for industry and government-funded operations. Surprisingly, though salmonid rearing density has been the subject of numerous studies, the observed results range from better

growth and condition at low densities (Kindschi and Koby 1994; Procarione et al. 1999; Wagner et al. 1997), to no significant density effects (Wagner et al. 1996). The majority of studies report some decrease in growth rate and overall condition as densities increase, but cutthroat-specific information is needed.

4. Conduct production-scale evaluations of the best diet × temperature × density combinations identified in the first three objectives. A common criticism of laboratory studies is that their results do not transfer to fish culture on a production scale. To address this issue, a comparison will be made between the growth of Snake River cutthroat trout reared under production conditions with the actual and predicted performance of the same subspecies when reared under laboratory conditions.
5. Develop outreach products to provide fish culturists and feed manufacturers with information on optimal growth temperatures, optimal rearing densities, and diet formulations for inland cutthroat trout subspecies. Although the commercial culture of cutthroat trout is not widespread, the species does offer a potential niche market, particularly in states where the stocking of native trout is preferred over non-native species. Therefore, providing growers with information on early culture techniques is a necessary step towards preparing them for this potential market.

## Materials and Methods

This study was completed at two different research institutions and one industry facility over the course of 4 years. The U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC) conducted the diet, temperature, and density experiments on the Snake River (SRCTT) and Yellowstone cutthroat trout (YCTT). The Fish Physiological Ecology Laboratory at Colorado State University (CSU) conducted the diet, temperature, and density experiments on the Colorado River cutthroat trout (CRCTT). Colorado State University also assisted with the production trial that was conducted at the Cline Trout Farms/Liley Fisheries facility in Colorado. Although the laboratory studies were conducted at different locations, a concerted effort was made to use similar rearing systems and techniques to allow qualitative comparisons between subspecies.

### *Rearing Systems and General Fish Husbandry*

Fish at BFTC and CSU were reared in rectangular aluminum troughs (122 cm × 36 cm × 23 cm) receiving a minimum of 3.7 L/min of flow-through air-saturated water at the desired temperature (see below). Water temperature fluctuations were kept to a maximum of ± 0.5°C through the use of manual and computer-controlled mixing valves; variation in temperature was generally less than ± 0.25°C.

Fish in all studies were fed diets using 12-h belt feeders; feeds were offered at a rate of 4% wt/d, a rate that proved to be an *ad libitum* ration, as evidenced by the presence of excess feed on the bottom of the tanks. Diet size (#0 - #3) was adjusted as necessary to match the fish sizes; when fish were of intermediate size, they were offered two different feed sizes. A subsample of 20 fish per tank was weighed and measured every 15 days to track intermediate growth; overall tank biomass was also taken at 15-d intervals (CSU) or 30-d intervals (BFTC) to allow adjustment of feed amounts. Four replicate tanks were used for each treatment (diet, temperature, temperature × diet, or density); tanks were used as the experimental unit because we did not mark fish in order to allow tracking of individuals. Cutthroat trout culture systems were kept on natural photoperiods, and tanks were cleaned as necessary (ca. daily).

Snake River cutthroat trout eggs were provided by the Jackson National Fish Hatchery; Yellowstone cutthroat trout eggs were provided by the Montana Department of Fish and Game, and; Colorado River cutthroat trout eggs were provided by the Colorado Division of Wildlife

Glenwood Springs Hatchery. All eggs were hatched at the research site and sac-fry were allowed to develop in incubation trays until swim-up, when they were transferred to the experimental tanks for the start of the diet, temperature, and density trials.

### *120-Day Diet Experiments*

A set of 120-day blind diet trials were conducted at BFTC on the Snake River and Yellowstone cutthroat trout, and at CSU on the Colorado River cutthroat trout. Investigators were provided with numbered diets for use in the study—a single individual at BFTC knew the identity of the diets. The diets used in this study were:

1. Rangen Regular Trout (RRT)
2. Rangen Soft Moist (RSM)
3. Silver Cup Regular Trout (SCRT)
4. Silver Cup Soft Moist (SCSM)
5. Skretting Nutra-Plus (now Bio-Vita Fry; SNP)
6. Skretting Nutra-Plus w/21 days of *Artemia nauplii* supplementation (CSU only; SNP+A)
7. Experimental 601 (E601)
8. Experimental 602 (E602).

All fish used in this study were first-feeding fry. The SRCTT were stocked at a density of 150 fish/tank; YCTT were stocked at 135 fish/tank, and both groups were reared at 10°C. The CRCTT were stocked at 150 fish/tank and were reared at 10.5°C. Each diet type was fed to four replicate tanks.

At the end of the 120-d diet trial, fish size and overall tank biomass were determined. Fulton's condition factor was determined using the wet weights and total length data. Survival, expressed as a percentage, was calculated from the final number of fish present in each tank. Kindschi's fin indices (Kindschi 1987) were used to quantify levels of fin erosion. One-way ANOVA were used to compare final fish wet weights, survival, and fin indices.

An additional analysis on the color of the fish was conducted at CSU following observations that fish fed different diets ranged from yellow to red as their dominant color. To test for differences in fin coloration produced by the diets, digital photographs were taken and adjusted as outlined by McGree (2008) Fish were lightly anesthetized before being photographed. Fish were placed on their left side, next to a color wheel, on a white background and photographed with a Kodak EasyShare CX7530 5.0MP digital camera. Photographs were manually adjusted for black and white in HP® Image Zone (Version 4.0) using the software's "shadow" and "highlight" tools on the black and white color wheel values, respectively.

A qualitative approach was used to analyze fin coloration because hatchery clientele decide what constitutes a colorful fish. Twenty observers were asked to view the 320 photographs (ten fish per tank;  $n = 40$  per treatment), in the same order, and rank the redness in the lower caudal and anal fins of each fish using a predetermined color rank scale (Figure 1.3). In the event that observers would become trained (i.e., better at ranking coloration) as they viewed the photos, photos were organized into ten strata and a photograph of one fish from each tank (four fish per treatment) was randomly placed within each strata. Thus, results should not be biased because any training that may have occurred should have been equalized amongst treatments.

When ranking redness, observers were allowed to use average scores of consecutive ranks (e.g., fins expressing colors in both the ranks of 1 and 2 could be scored as a 1.5), but were not

allowed to use averages of non-consecutive ranks (e.g., ranks of 1 and 4 could not be scored as 2.5). In the case where an observer viewed colors from non-consecutive ranks they were asked to determine the most prominent color present and assign a rank accordingly.

#### *65-day Diet Experiment*

An additional 65-d diet trial was performed at the BFTC to compare the effects of different diet supplements on the growth, survival, and performance of SRCTT. The supplements used were the following: Skretting/Bio-Oregon Bio-Vita (SNP), Skretting/Bio-Oregon Bio-Vita + dry *Artemia* flake (SNP+DA), Skretting/Bio-Oregon Bio-Vita + freeze dried cyclopeeze (SNP+C), Silver Cup Fry Micro Pellet Salmon (SCFMP), Otohime (OTH), Otohime + freeze dried cyclopeeze (OTH+C), and Silver Cup Regular Trout (SCRT). Procedures used in this trial were as described above, except fish were held at 14.5°C and the experiment was only run for 65 days.

#### *Water Temperature Experiment*

The effects of water temperature on the survival, growth, and performance of first-feeding cutthroat trout were measured in a series of 120-d growth studies. Snake River and Yellowstone cutthroat trout were reared at 10, 12, 14, 16, 18, and 20°C at BFTC. These fish were fed 4% wt./d rations of SNP, which was selected because it provided the highest growth rates in the diet experiments conducted previously. Colorado River cutthroat trout were reared in a 2 × 5 factorial study comparing the effects of temperature (10, 12.5, 15, 17.5, and 20°C) and diet (SNP+A) and Rangen Regular Trout, supplemented with live *Artemia* for 21 days (RRT+A) because the results from the previous diet study identified two possible diets as candidates for the “best” diet. Unfortunately, a system failure at CSU in the middle of the temperature experiment forced the termination of the 10°C treatment on CRCTT, so only final size data from the 12.5 to 20°C treatments were collected.

Fish husbandry, data collection, and data analyses were as described above, with the following changes. Color information was not collected on fish at CSU. Additionally, 2-way ANOVA were used to compare the effects of diet and temperature and the diet × temperature interaction for the CRCTT. Finally, quadratic regression equations were fitted to the temperature and final wet weight data to identify the optimal growth temperatures.

#### *Rearing Density Experiment*

Snake River and Yellowstone cutthroat trout were reared at initial densities of 50, 100, 150, 200, 250, 300, and 350 fish/tank at 14.5°C (optimal temperature identified in the prior experiment) to study the effects of rearing density on growth, survival, and performance. Colorado River cutthroat trout were reared at densities of 150, 300, 450, and 600 fish/tank<sup>8</sup> at the experimentally-determined optimum of 16.4°C. All fish were fed at 4% wt./d.

Fish husbandry, data collection, and data analyses were as described above, with the following changes. No color information was collected or analyzed. Comparisons of final wet weight and survival as a function of density were made using one-way ANOVA.

#### *Production Scale Growth Experiment*

A production-scale growth trial using Snake River cutthroat trout was conducted at the Liley Fisheries facility (formerly the Cline Trout Farms Boulder Facility). First-feeding Snake River cutthroat trout were held in shallow, rectangular hatchery troughs at ambient well water temperatures (13 – 14°C during the course of the study) and were fed SNP at a maximum of 4%

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<sup>8</sup> Higher densities were used at CSU following consultation with BFTC and project workgroup personnel; the results from the BFTC density trials indicated that while differences were observed, they were small, so an effort was made to increase densities to a level where a stronger density effect would be observed.

wt./d over a 120-d period. Other than the specialized diet, these cutthroat trout were handled using the same techniques that would be used for rainbow trout at this facility. The goal of this experiment was to see how closely the actual growth of the fish under production conditions matched the predicted growth of SRCTT based on the results previously found in this project. The SRCTT growth predictions were made using the temperature × growth equations developed from the results of the temperature experiments.

#### *Development of Outreach Products*

The project team felt that it was desirable to provide regular updates on project progress and findings to industry and agency/academic audiences. This was primarily done through an ambitious schedule of presentations to industry and agency/academic conferences and through periodic publications. It was also determined that a project website would be developed and hosted by the Western Regional Aquaculture Center to provide a central location where project information could be accessed. Finally, the primary outreach product for this study was to be a manual on cutthroat trout production that would synthesize information developed by the study along with information from other sources (e.g., interviews of current cutthroat trout producers).

### **Results**

#### *120-Day Diet Experiments*

Diet significantly affected Yellowstone cutthroat fish wet weight, total length, survival, and feed conversion ratios, but did not affect the dorsal fin index (Table 1). The diet that produced the largest fish (3.73 g/fish) was SNP, while the smallest fish were those fed RSM. Although there were differences in survival, these were small, and survival was uniformly high for all treatments (> 89%). A similar result was observed for Snake River cutthroat trout, with significant diet effects on fish wet weight, total length, and food conversion, but no diet effect on survival or fin condition (Table 2).

Diet type significantly affected the growth and survival of the first-feeding Colorado River cutthroat trout, but did not affect their fin quality (as determined by the dorsal fin index). Cutthroat trout fed SNP+A were significantly heavier, longer, and had better overall survival than any of the other Diet treatments (Table 3). This indicates that supplementing the prepared diet with live *Artemia* nauplii for 21 days of feeding confers a significant benefit to the fish at a relatively low cost. Similar results have been reported by Arndt and Wagner (2007) in a recent study on the same cutthroat trout subspecies. If the *Artemia*-supplemented diet is excluded, then RRT, SCRT, SNP, and E601 Diets #1, #3, #5, and #6 produced fish of similar weight, but only RRT and SNP produced the longest fish that also, coincidentally, had the highest survival rate (Table 3). The condition of the fish was uniformly good, as indicated by the lack of statistically significant differences among treatments' dorsal fin index scores.

One area of interest that was not included in the original proposal was an assessment of the difference in the coloration of the CRCTT. The diets produced a striking gradation in fin coloration, from pale to red colored fins (Figure 2). There were significant differences in coloration ranks between fish fed the eight diets (Figure 3). Experimental 601, a diet supplemented with astaxanthin, produced fish with significantly more red in their fins than fish fed the other seven diets. Skretting nutra-plus and the *Artemia*-supplemented diet produced fins orange to red in color, and Rangen regular trout and Silver Cup soft-moist produced orange colored fins. Fish fed Rangen soft-moist and Experimental 602 had significantly paler fins than fish fed the other six diets.

#### *65-Day Diet Experiment*

The results of this additional diet trial demonstrated that other starter diets (both premium and

non-premium) produced the same high levels of survival (> 93%) with SRCTT as did SNP (Figure 4). Additionally, the difference in weight between the fish fed SNP and those fed SNP+A, SNP+DA, SNP+C, OTH, or OTH+C were not significant, indicating that, for SRCTT at least, there is no clear benefit to diet supplementation, and, more importantly, that the use of any typical premium diet provides a similar level of growth performance.

#### *Effects of Water Temperature on Cutthroat Trout*

Water temperature significantly affected ( $P < 0.001$ ) the final fish size for both SRCTT (Figure 5) and YCTT (Figure 6), but did not affect the survival or fin condition of either subspecies. The optimal temperature for growth of first-feeding SRCTT was calculated to be 14.5°C. The regression expression for final fish wet weight on day 120 as a function of water temperature ( $T_W$ ) is shown below.

$$\text{Wet weight}_{\text{Day } 120} = 4.894 - (0.5089 \times T_W) - 0.0653(T_W - 14.9167)^2 \quad (R^2: 0.92)$$

The optimal temperature for growth of YCTT was calculated to be 14.7°C, and the regression expression for final fish wet weight as a function of water temperature is:

$$\text{Wet weight}_{\text{Day } 120} = 6.096 - (0.0414 \times T_W) - 0.0892(T_W - 14.9)^2 \quad (R^2: 0.93)$$

Diet, temperature, and the diet × temperature interaction all had significant effects on CRCTT wet weight and total length (Table 4). For all temperatures, fish fed SNP+A were longer, heavier, and had higher survival than those fed RRT+A, thereby confirming that SNP+A was the best diet of those tested for CRCTT. Quadratic regressions (Figure 7) indicated that the optimal temperatures for growth were 15.3°C for fish fed RRT+A and 16.4°C for fish fed SNP+A. The regression expressions for fish fed RRT+A and SNP+A are shown below.

$$\text{RRT+A Wet weight}_{\text{Day } 120} = 30.417 - (4.9330 \times T_w) - (0.1612 \times T_w^2) \quad (R^2 = 0.99)$$

$$\text{SNP+A Wet weight}_{\text{Day } 120} = 59.252 + (8.5692 \times T_w) - (0.2616 \times T_w^2) \quad (R^2 = 0.90)$$

Survival was unaffected by diet or by the diet × temperature interaction, but temperature did have a significant, inverse relationship with survival (Table 4) wherein fish reared at temperatures of 15°C or less had higher survival than those reared at temperatures exceeding 15°C.

#### *Rearing Density Experiment*

Initial stocking density had a significant effect on the growth of Snake River, Yellowstone (Table 5), and Colorado River cutthroat trout (Table 6; linear regression;  $P < 0.05$ ), with lower final fish wet weights at the higher densities. Survival of Snake River cutthroat trout did show a slight, but statistically significant decline as density increased, but the survival of YCTT and CRCTT was unaffected by density. Fin condition was also unaffected by density at the levels tested.

#### *Production Trial*

Using the equation developed from the SRCTT data in the temperature experiments, we predicted that the SRCTT reared at 13 – 14°C in the production trial should have mean final wet weights (day 120) between  $3.89 \pm 0.13$  g and  $4.01 \pm 0.13$  g (mean ± SE). Although this is only a qualitative comparison (the nature of the production trial did not allow for quantitative analyses), the mean final wet weight of the fish ( $3.62 \pm 1.13$  g) falls within the expected range (Figure 8).

#### *Development of Outreach Products*

The project team has actively sought venues to present the most recent findings of the cutthroat trout study to industry and professional audiences, both in oral and written format. A total of 7 presentations to professional fisheries society meetings were made during the project period, with an additional 7 presentations delivered to aquaculture industry conferences. There have

also been at least three publications (1 peer reviewed) prepared, including a M.S. thesis by the graduate student at CSU, Ms. Mandi Brandt.

The project website has been ready for hosting since November 2006 (Figure 9), though difficulties in having WRAC host the website have delayed the actual release of the information. The recent version of the WRAC website does include some information about the project, so hopefully this will soon be updated to include much of the information produced on this project.

The primary outreach component of the project, a WRAC Extension publication on cutthroat trout culture, is currently being written by the Outreach Coordinator and Workgroup Chair. Progress to date includes the completion of drafts of 3 of the 5 planned sections. Once the remainder of the manual is completed, it will be submitted to the WRAC Extension review committee before final submission to the IAC/TC.

## Discussion

The multi-institution study on the effects of diet, temperature, and rearing density on the survival, growth, and performance of first-feeding cutthroat trout met the overall goals of identifying optimal diets, thermal regimes, and rearing densities for the successful culture of the species. Additionally, the study demonstrated that the data collected under laboratory conditions for Snake River cutthroat trout could be used to predict the performance of the same subspecies when reared under production hatchery conditions.

One goal of the study was to identify currently available diets that could be used to improve the growth and survival of first-feeding cutthroat trout. The diet that proved most suitable for Snake River, Yellowstone, and Colorado River cutthroat trout was SNP, which is a premium salmon starter diet. Interestingly, a follow-up study that looked at other premium starter diets showed that they generally produced the same level of performance and growth, underscoring the importance of relying on high-quality feeds for sensitive life stages.

An additional finding that should be of interest to fish culturists raising juvenile cutthroat trout is that of the increased growth and survival of CRCTT that were fed *Artemia* nauplii for the first 21 days. Even after another 98 days had elapsed, the difference in size and survival was still apparent, clearly indicating that the use of live feed conferred a significant and lasting advantage to these fish. Similar increases in growth have been reported in other species including shortnose sturgeon (*Acipenser brevirostrum*) (Ware et al. 2006), possibly because of the nutritional value, high palatability, and greater motility of the nauplii relative to slowly sinking feed particles (Arndt and Wagner 2007). The primary drawback to using live *Artemia* is the added cost and time involved with culturing the organism, so it was encouraging to see that the use of diets combined with various dried supplements (*Artemia*, Cyclopeeze) did not produce significantly different SRCTT growth or survival rates when compared with a diet supplemented with live *Artemia*. The difference in response between the CRCTT and SRCTT may reflect the different nature of the broodstocks. The CRCTT eggs were collected annually from wild broodstock found in Nanita Lake, Colorado, so presumably these fish were not as well adapted to hatchery conditions as are the SRCTT, which were produced from eggs collected from captive broodstock held at the Jackson National Fish Hatchery. It is possible that a reduced reliance on live feed could result through the development of captive CRCTT broodstock.

The influence of the diets on CRCTT coloration should also be noted. While fish color was not one of the factors initially considered as important, it stands to reason that clients purchasing fish for stocking in private waters would be interested in fish that displayed more vivid colors, presumably because of their greater appeal to anglers. The use of carotenoids such as astaxanthin and canthaxanthin to produce colorful fish is well-established in aquaculture, and producers may want to consider using feeds with these supplements to increase the visual

attractiveness of their products.

The results of the water temperature experiments identified the optimal temperatures for the growth of Snake River, Yellowstone, and Colorado River cutthroat trout fed to satiation on a premium starter diet. It should be noted that while the closely-related YCTT and SRCTT had optimal temperatures of 14.5 – 14.7°C, the optimal temperature found for CRCTT fed the same diet (SNP) was almost 2°C higher. The optimal temperature of another closely-related subspecies, the westlope cutthroat trout, is slightly lower (13.6°C) (Bear et al. 2007), while the optimal temperature for rearing rainbow trout has been reported as falling between 15 and 19°C, depending on feeding rate, rearing density, and water quality (Myrick and Cech 2004).

The difference between optimal growth temperatures for CRCTT and the two subspecies reared at BFTC (and westslope cutthroat trout, another subspecies from that region) could stem from slightly difference culture conditions at BFTC and CSU, but it is more likely that this reflects an evolutionary difference among the subspecies. Colorado River cutthroat trout historically occurred at lower latitudes than either YCTT or SRCTT (Behnke 1992) and thus would have the opportunity to take advantage of a longer growing season and potentially higher summer water temperatures. Having a higher optimal growth temperature would allow this subspecies to grow more rapidly during this window of opportunity. Ironically, it is currently rare for stream reaches that still contain CRCTT to reach 16.4°C, even during the summertime, because the fish are now largely restricted to high-elevation streams and lakes (Hirsch et al. 2006; Young et al. 1996).

The value of the predictive equations developed for the three subspecies was succinctly illustrated by the use of the SRCTT equation to predict the size of fish in a production setting. These equations could be used, albeit cautiously, to make predictions of fish production rates in hatchery environments. It should be noted, however, that the equations apply only to fish that are being fed to satiation on a premium diet; as has been noted by other authors, fish that are fed at a reduced rate or that are fed a diet of lower quality will have lower optimal growth temperatures (Fiogbé and Kestemont 2003; Jobling 1994).

The results of the rearing density experiments showed that slight, but significant reductions in growth were associated with the highest densities of SRCTT, YCTT, and CRCTT. The reductions in growth are likely not the result of poor water quality because both BFTC and CSU maintained high dissolved oxygen concentrations in the culture tanks (e.g., the effluent DO concentration in the 600 fish/tank treatments at CSU never dropped below 7.20 mg/L), and flows were high enough to keep total ammonia nitrogen levels below detection limits. Thus, the most likely cause of the reduced growth was the effect of crowding, though, interestingly, there was not a corresponding increase in fin erosion or damage, which is similar to what Wagner et al. (Wagner et al. 1997) observed in Bear Lake strain Bonneville cutthroat trout (*O. c. utah*). Based on these results, it appears that rearing cutthroat trout at densities exceeding 250 – 300 fish/tank could prove counterproductive, so the fish should be thinned out as necessary to avoid depressing the growth rates, particularly as fish approach the 120 days post swim-up.

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**Tables**

Table 1. Mean performance of Yellowstone cutthroat trout fed one of seven diets at the Bozeman Fish Technology Center. Means (n = 4, pooled SEM) in the same column with no or the same superscript are not significantly different (P>0.05). Please see the text for descriptions of the diet abbreviations.

Diet	Final Weight (g/fish)	Final Total Length (mm)	Survival (%)	Feed Conversion	Dorsal Fin Index (%)
RRT	2.74 <sup>x</sup>	68.4 <sup>x</sup>	93.6 <sup>zy</sup>	1.95 <sup>z</sup>	10.0
RSM	2.17 <sup>w</sup>	60.9 <sup>w</sup>	89.2 <sup>y</sup>	2.04 <sup>z</sup>	10.1
SCRT	3.17 <sup>y</sup>	73.2 <sup>zy</sup>	92.5 <sup>y</sup>	1.85 <sup>zy</sup>	10.4
SCSM	2.94 <sup>yx</sup>	70.4 <sup>yx</sup>	93.2 <sup>zy</sup>	1.75 <sup>zy</sup>	10.9
SNP	3.73 <sup>z</sup>	75.7 <sup>z</sup>	97.8 <sup>z</sup>	1.64 <sup>y</sup>	10.5
E601	2.74 <sup>x</sup>	69.1 <sup>x</sup>	91.3 <sup>y</sup>	1.85 <sup>zy</sup>	10.1
E602	2.84 <sup>x</sup>	69.3 <sup>x</sup>	92.0 <sup>y</sup>	1.84 <sup>zy</sup>	10.6
SEM	0.18	1.75	1.0	0.05	0.12

Table 2. Mean performance of Snake River cutthroat trout fed one of seven diets at the Bozeman Fish Technology Center. Means (n = 4, pooled SEM) in the same column with no or the same superscript are not significantly different (P>0.05) Please see the report text for descriptions of the diet abbreviations

Diet	Final Weight (g/fish)	Final Total Length (mm)	Survival (%)	Feed Conversion	Dorsal Fin Index (%)
RRT	3.56 <sup>w</sup>	71.8 <sup>x</sup>	97.6	1.80 <sup>z</sup>	9.9 <sup>yw</sup>
RSM	2.77 <sup>v</sup>	65.7 <sup>w</sup>	95.2	1.62 <sup>zy</sup>	9.7 <sup>xw</sup>
SCRT	4.03 <sup>y</sup>	77.3 <sup>y</sup>	96.1	1.66 <sup>zy</sup>	10.7 <sup>z</sup>
SCSM	3.76 <sup>x</sup>	73.6 <sup>x</sup>	96.4	1.59 <sup>zy</sup>	10.1 <sup>zyw</sup>
SNP	4.78 <sup>z</sup>	80.9 <sup>z</sup>	97.7	1.54 <sup>y</sup>	10.4 <sup>zy</sup>
E601	3.78 <sup>x</sup>	74.6 <sup>x</sup>	97.4	1.63 <sup>zy</sup>	9.5 <sup>wv</sup>
E602	3.82 <sup>x</sup>	72.9 <sup>x</sup>	94.3	1.55 <sup>y</sup>	9.0 <sup>v</sup>
SEM	0.23	1.79	0.49	0.03	0.22

Table 3. Summary of the effects of diet type on the survival, growth, and condition of Colorado River cutthroat trout fed 8 experimental diets over a 120-d diet trial. Values are means  $\pm$  SEM. Means in the same column followed by different superscripted letters are significantly different (ANOVA,  $P < 0.05$ ). The mean size of the fish at the start of the diet trial was  $0.07 \pm 0.014$  g.

Diet	Final Weight (g/fish)	Final Total Length (mm)	Survival (%)	Dorsal Fin Index (%)
RRT	$4.20 \pm 0.13^b$	$77.9 \pm 0.76^{bc}$	$78.5 \pm 0.82^{ab}$	$15.8 \pm 0.24^a$
RSM	$1.83 \pm 0.08^d$	$58.7 \pm 0.83^f$	$73.5 \pm 3.76^{ab}$	$15.0 \pm 0.29^a$
SCRT	$3.80 \pm 0.16^b$	$75.6 \pm 1.00^c$	$68.8 \pm 0.64^{bc}$	$15.3 \pm 0.17^a$
SCSM	$2.24 \pm 0.10^d$	$63.5 \pm 1.02^e$	$55.9 \pm 1.83^c$	$15.7 \pm 0.23^a$
SNP	$3.89 \pm 0.14^b$	$77.5 \pm 0.74^b$	$80.0 \pm 1.91^{ab}$	$15.1 \pm 0.18^a$
SNP+A	$5.12 \pm 0.13^a$	$83.2 \pm 0.64^a$	$86 \pm 2.23^a$	$15.4 \pm 0.14^a$
E601	$3.90 \pm 0.13^b$	$75.4 \pm 0.87^c$	$68.0 \pm 2.35^{bc}$	$15.3 \pm 0.22^a$
E602	$3.10 \pm 0.14^c$	$69.8 \pm 1.01^d$	$70.4 \pm 6.83^{bc}$	$15.8 \pm 0.23^a$

Table 4. Summary of the effects of diet type and temperature on the growth, condition, and survival of Colorado River cutthroat trout. In the final weight and final total length columns diet  $\times$  temperature interaction effects are shown using letters and temperature effects are shown using asterisks. Means in the same column followed by different superscripted letters or different numbers of asterisks are significantly different (ANOVA,  $P < 0.05$ ). The superscripted letters in the survival column indicate temperature effects. There was no statistically significant diet, temperature, or diet  $\times$  temperature interaction for dorsal fin index.

Diet $\times$ Temperature Treatment	Final Weight $\pm$ SEM (g)	Final Total Length $\pm$ SEM (mm)	Survival $\pm$ SEM (%)	Dorsal Fin Index $\pm$ SEM (%)
RRT+A – 12.5°C	$6.09 \pm 0.53^{c**}$	$84.0 \pm 5.15^{c**}$	$51.7 \pm 4.59^{ab}$	$10.7 \pm 0.10$
RRT+A – 15.0°C	$7.21 \pm 0.75^{c*}$	$87.4 \pm 6.56^{c*}$	$45.7 \pm 4.27^{bc}$	$10.8 \pm 0.10$
RRT+A – 17.5°C	$6.64 \pm 0.65^{c*}$	$84.4 \pm 6.02^{c*}$	$36.2 \pm 3.30^{bc}$	$10.8 \pm 0.10$
RRT+A – 20.0°C	$3.73 \pm 0.30^{d**}$	$69.7 \pm 3.88^{d**}$	$39.3 \pm 4.70^c$	$10.9 \pm 0.10$
SNP+A – 12.5°C	$7.24 \pm 0.57^{c**}$	$89.0 \pm 5.43^{c**}$	$55.2 \pm 3.55^{ab}$	$10.9 \pm 0.10$
SNP+A – 15.0°C	$9.67 \pm 0.82^{b*}$	$97.2 \pm 5.62^{b*}$	$52.3 \pm 4.32^{bc}$	$11.0 \pm 0.10$
SNP+A – 17.5°C	$11.35 \pm 0.86^{a*}$	$101.4 \pm 5.19^{a*}$	$46.8 \pm 4.90^{bc}$	$11.0 \pm 0.10$
SNP+A – 20.0°C	$7.24 \pm 0.47^{c**}$	$86.4 \pm 4.49^{c**}$	$40.3 \pm 7.25^c$	$10.7 \pm 0.10$

Table 5. Effects of density on the final size (g/fish), and survival (%), and condition factor (K) of Snake River and Yellowstone cutthroat trout. Values are means  $\pm$  SEM. There were 4 tanks per treatment.

Cutthroat Trout subspecies	Density Treatment (Number of Fish)	Final wet weight (g/fish)	Survival (%)	Condition Factor (K)
Snake River	50	3.0 $\pm$ 0.14	97.5 $\pm$ 0.50	90.0 $\pm$ 3.74
	100	2.8 $\pm$ 0.05	96.8 $\pm$ 1.25	91.5 $\pm$ 1.71
	150	2.8 $\pm$ 0.12	95.5 $\pm$ 1.26	86.0 $\pm$ 8.21
	200	2.8 $\pm$ 0.06	95.2 $\pm$ 1.20	89.0 $\pm$ 0.58
	250	2.6 $\pm$ 0.03	92.7 $\pm$ 1.19	88.7 $\pm$ 3.18
	300	2.5 $\pm$ 0.08	93.4 $\pm$ 0.71	82.2 $\pm$ 4.30
	350	2.5 $\pm$ 0.07	91.7 $\pm$ 2.01	84.2 $\pm$ 0.48
Yellowstone	50	5.3 $\pm$ 0.21	95.0 $\pm$ 2.08	80.5 $\pm$ 0.29
	100	4.9 $\pm$ 0.16	95.0 $\pm$ 1.22	85.0 $\pm$ 1.47
	150	4.8 $\pm$ 0.21	97.3 $\pm$ 0.71	80.5 $\pm$ 2.99
	200	4.78 $\pm$ 0.15	96.4 $\pm$ 0.72	83.8 $\pm$ 2.75
	250	4.5 $\pm$ 0.03	96.3 $\pm$ 1.17	76.2 $\pm$ 1.65
	300	4.5 $\pm$ 0.12	96.5 $\pm$ 1.44	78.5 $\pm$ 1.19
	350	4.6 $\pm$ 0.09	94.9 $\pm$ 0.65	86.0 $\pm$ 3.0

Table 6. Mean final total length (TL), mean final wet weight (WW), and condition factors of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) following a 120-d rearing density experiment. Data are expressed as mean  $\pm$  SEM; values with different letters indicate statistical significance (one-way ANOVA tests;  $\alpha = 0.05$ ).

Rearing density (fish/tank)	Somatic growth		Condition Factor (K)
	TL (mm)	WW (g)	
150	105 $\pm$ 1.2 z	11.92 $\pm$ 0.41 zy	0.99 $\pm$ 0.01 z
300	106 $\pm$ 2.0 z	12.06 $\pm$ 0.74 z	0.97 $\pm$ 0.01 zy
450	102 $\pm$ 1.1 z	10.38 $\pm$ 0.36 y	0.96 $\pm$ 0.01 y
600	94 $\pm$ 1.2 y	8.07 $\pm$ 0.31 x	0.92 $\pm$ 0.01 x

**Figures**

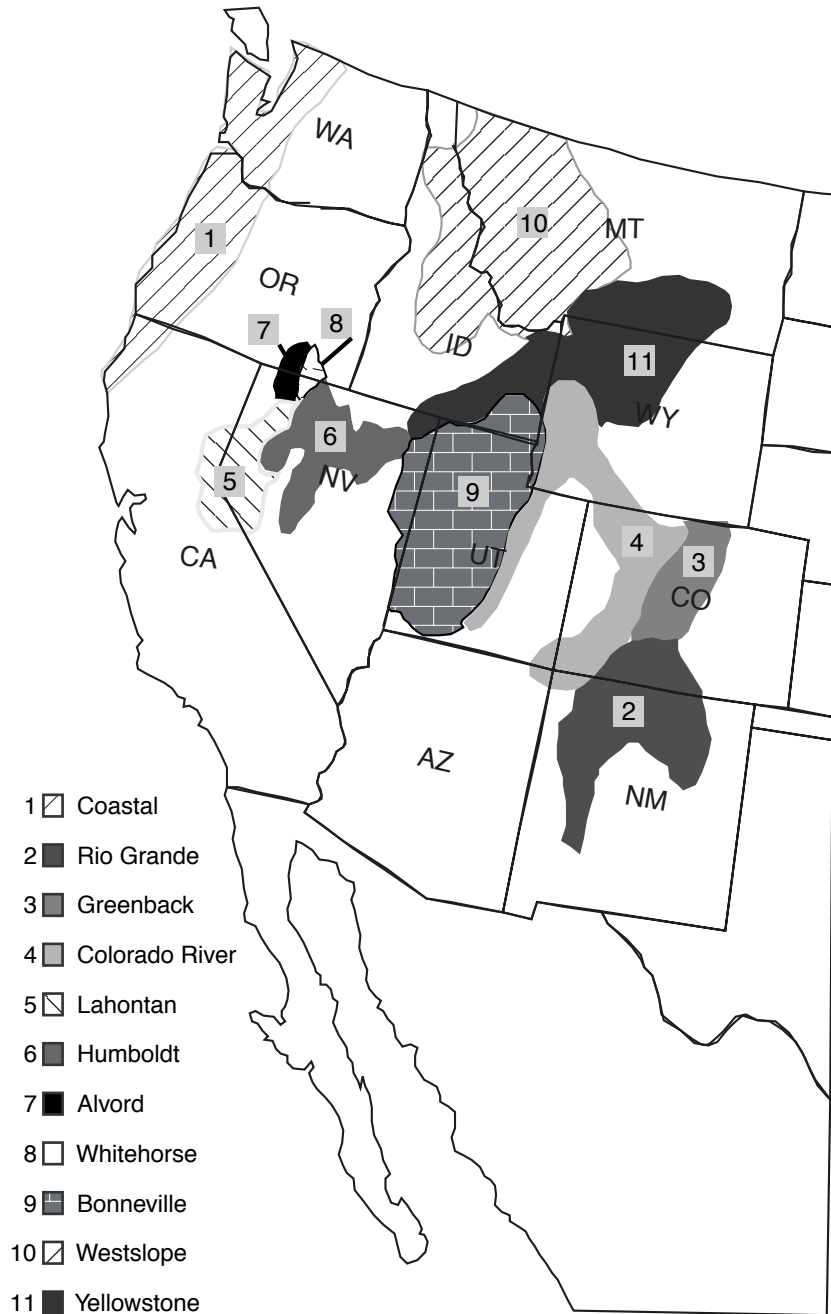


Figure 1. Map showing the distribution of extant cutthroat trout subspecies in the western United States. Data are from Behnke (1992). The Snake River subspecies is not shown because it has been very widely introduced throughout this region.

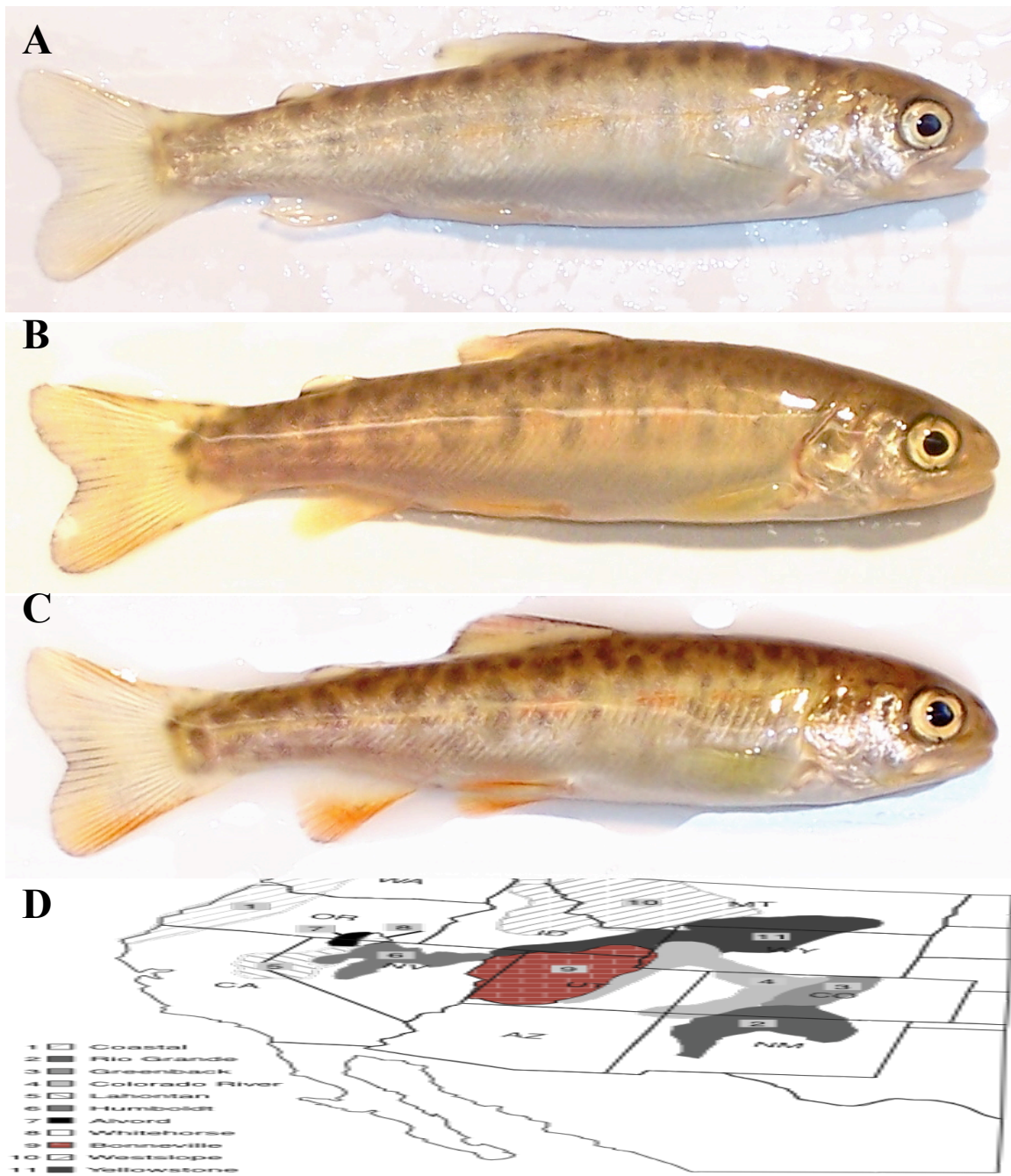


Figure 2. Fin coloration of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) produced by eight diets used in a 119-day feeding trial. Fins ranged from pale colored (A), to yellow (B), to orange (C), to red (D).

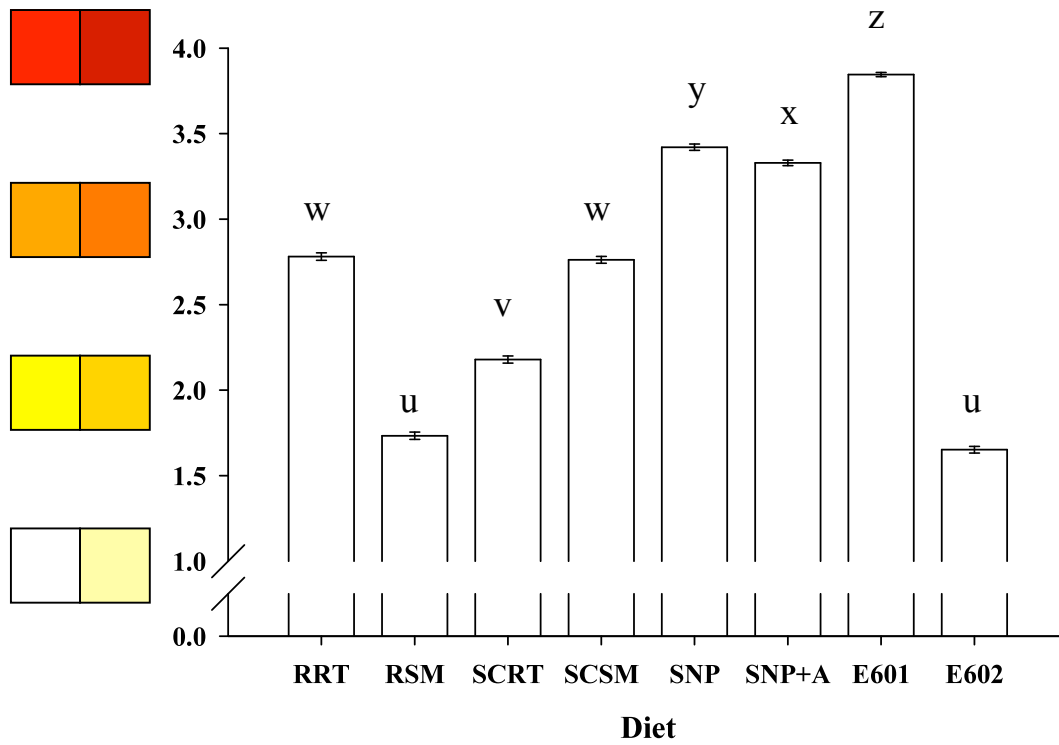


Figure 3. Fin coloration ranks of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) following a 119-d feeding trial. There were significant differences in coloration ( $p < 0.0001$ ). Data are expressed as means  $\pm$  SEM; values with different letters indicate statistical significance (one-way ANOVA test;  $\alpha = 0.05$ ,  $n = 40$  per treatment).



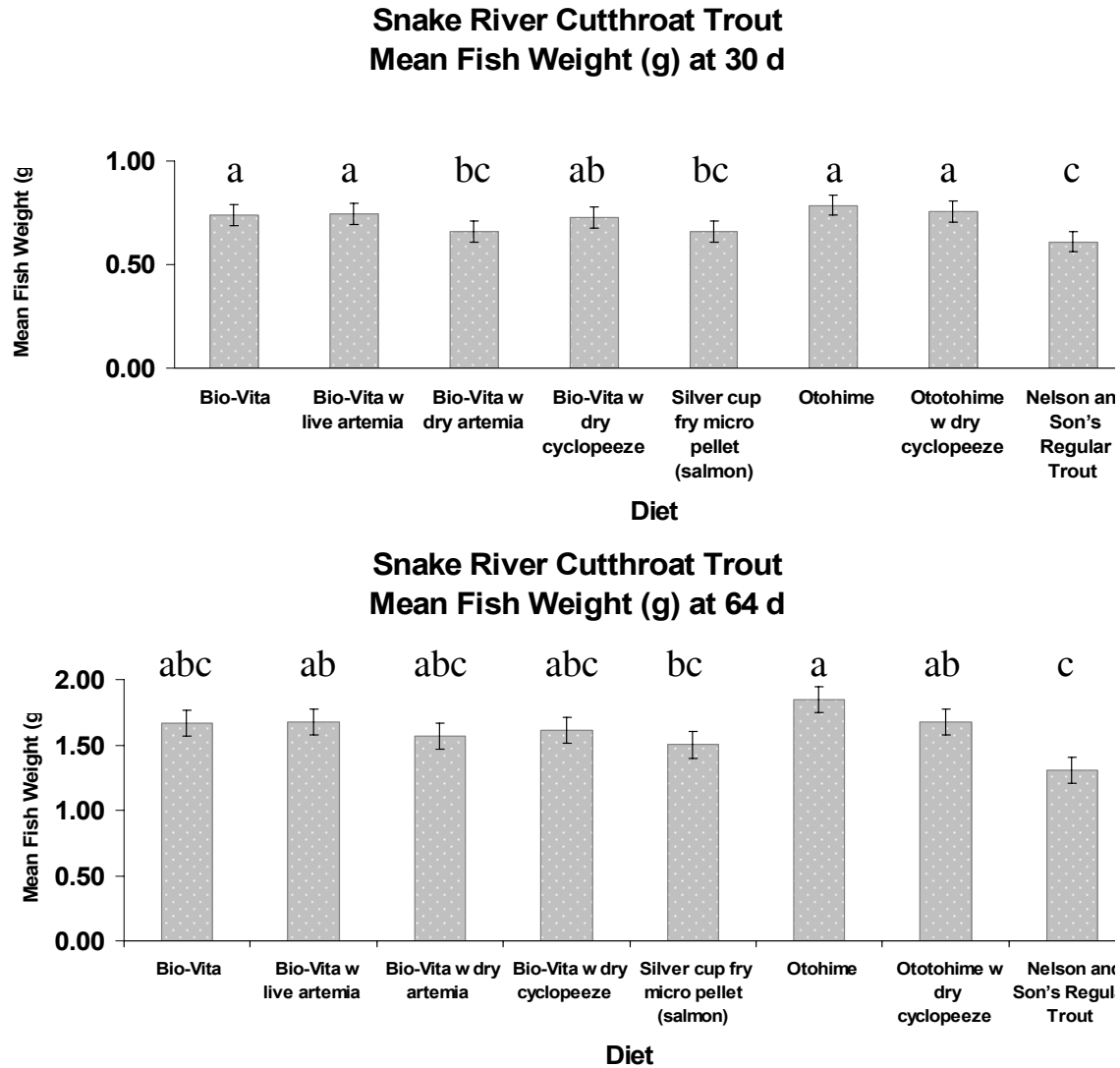


Figure 4. Effects of diet type and supplementation regimes on the final wet weight of juvenile Snake River cutthroat trout. Data are expressed as means  $\pm$  SEM; values with different letters indicate statistical significance (one-way ANOVA test;  $\alpha = 0.05$ ).

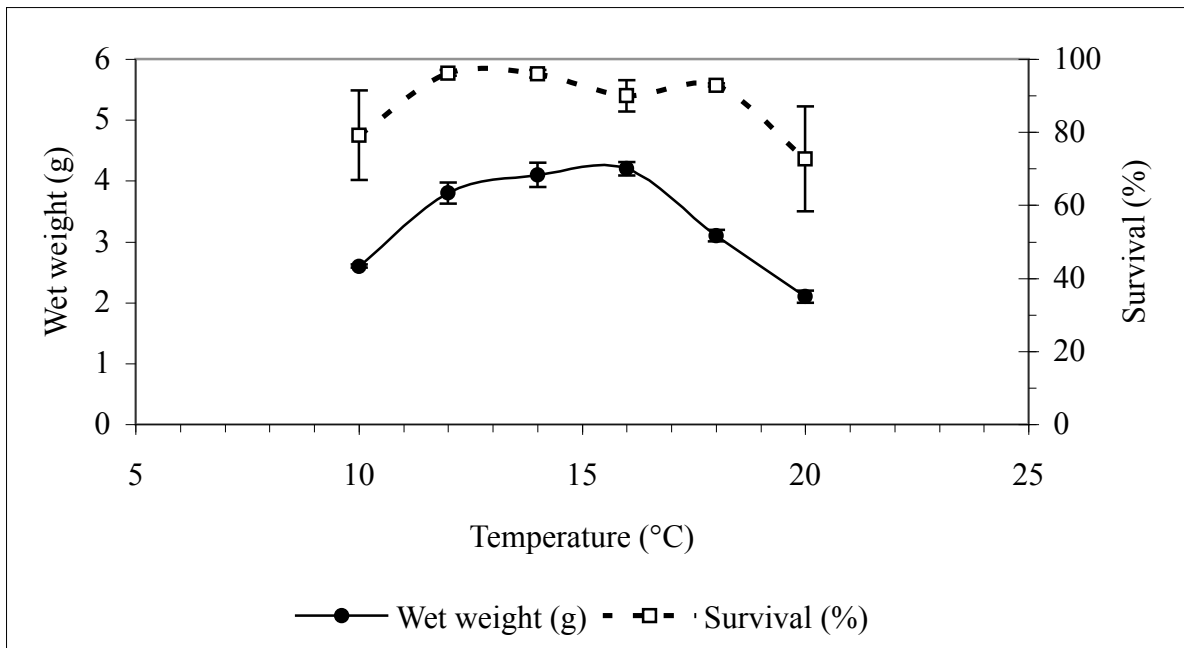


Figure 5. Effects of temperature on the growth and survival of Snake River cutthroat trout. The optimal temperature for growth was estimated to be 14.5°C ( $R^2: 0.92, P < 0.001$ ). Temperature significantly affected SRCTT growth rate, but not survival. Error bars are SEM.

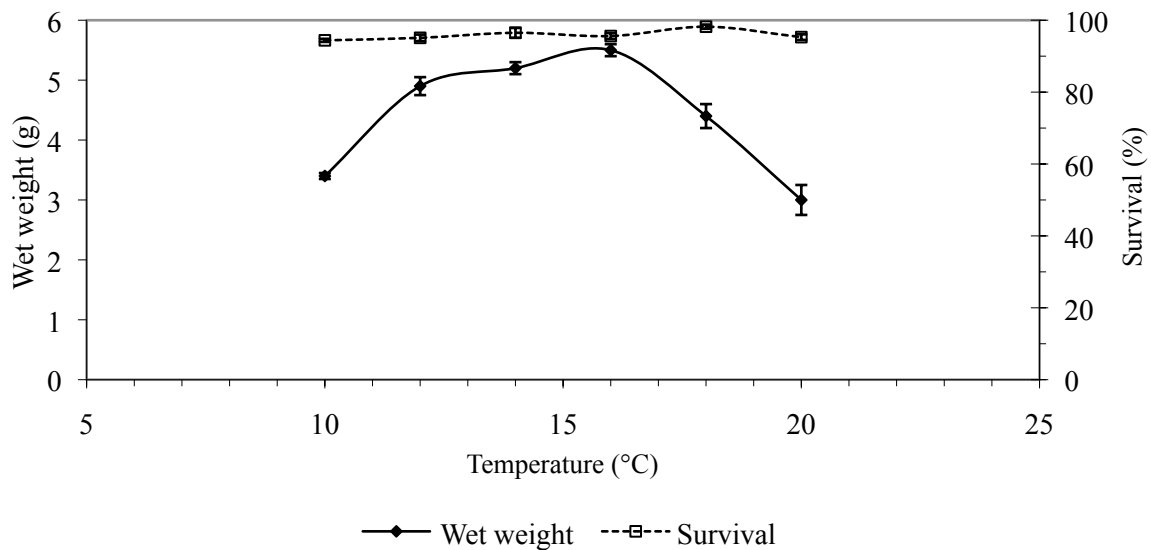


Figure 6. Effects of temperature on the growth and survival of Yellowstone cutthroat trout. The optimal temperature for growth was estimated to be 14.7°C ( $R^2: 0.93; P < 0.001$ ). Water temperature had a significant ( $P < 0.001$ ) effect on fish size, but did not significantly affect survival. Error bars are SEM.

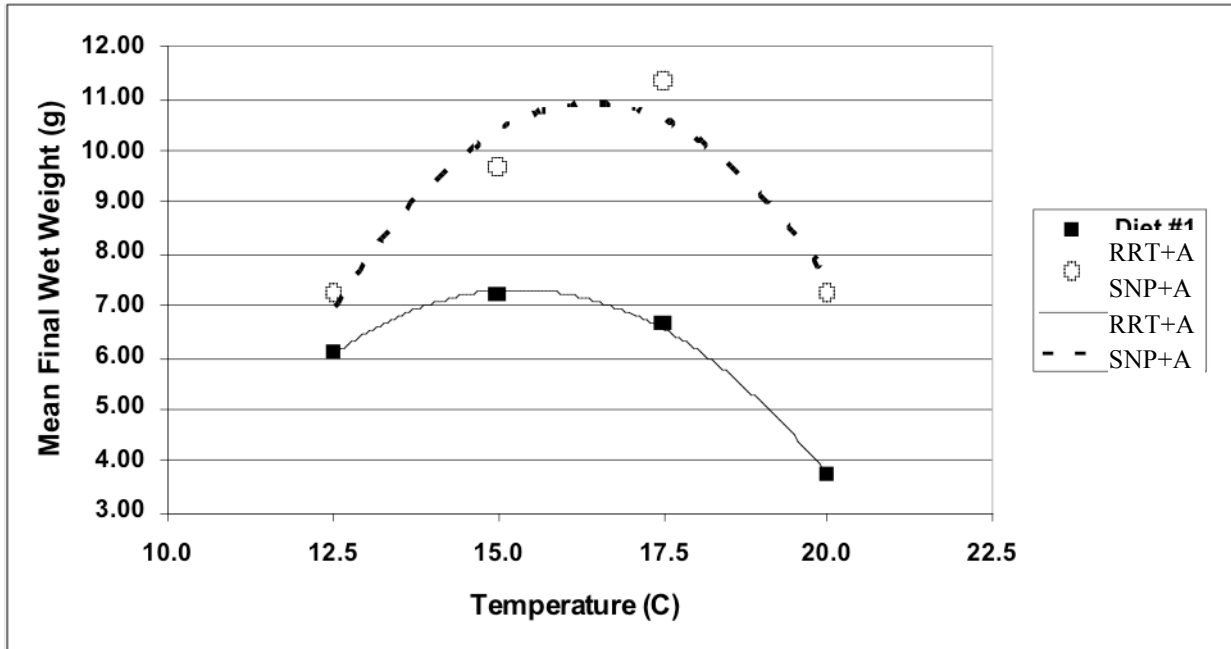


Figure 7. Quadratic regressions showing the optimal rearing temperatures for Colorado River cutthroat trout growth in terms of mean final wet weight (g) for RRT+A (15.3°C) and SNP+A (16.4°C).

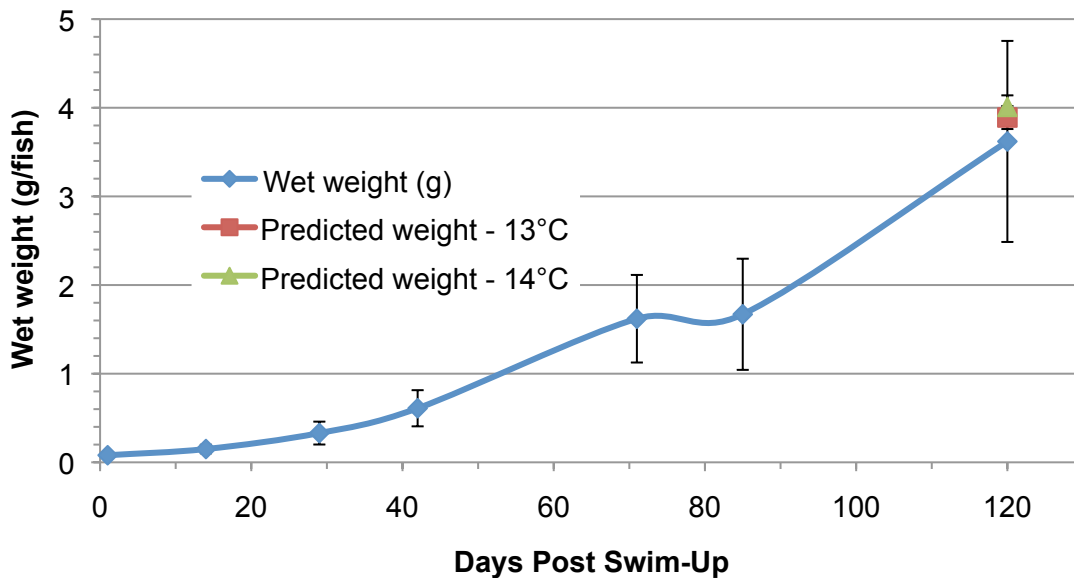


Figure 8. Growth over time of Snake River cutthroat trout fed the SNP diet under hatchery production conditions. The figure also provides a qualitative comparison of actual and predicted wet weights of Snake River cutthroat trout reared under production conditions using the SNP diet. The mean final wet weight of fish in the production trial was  $3.62 \pm 1.13$  g (mean  $\pm$  SE) and the predicted ranges for fish reared at 13 and 14°C were  $3.89 \pm 0.13$  g and  $4.01 \pm 0.13$  g, respectively.



Figure 9. Screenshot of the home page of the website that was developed for the cutthroat and Gila trout culture project in November 2006. The purpose of the website was to provide a central location where interested stakeholders could access the annual reports and presentations prepared by the project team.

**IMPACTS:****Development of culture conditions for first-feeding cutthroat trout**

**Relevance:** In 2004, the aquaculture industry in the Western United States requested a comprehensive research project on the development of culture conditions (diet, temperature, rearing density) for first-feeding cutthroat trout of various subspecies. This request was made because past attempts to culture cutthroat trout using diets and techniques developed for rainbow trout had not been very successful.

**Response:** From 2005 through 2009, a multi-institution team conducted a series of experiments on the effects of diet type, water temperature, and rearing density on the survival, growth, and performance of first-feeding Snake River, Colorado River, and Yellowstone cutthroat trout. An additional production-scale experiment was conducted to verify that results from laboratory studies could be adequately transferred to production-level efforts.

**Results:** The study demonstrated that first-feeding cutthroat trout have the highest growth rates and survival when fed premium trout or salmon diets like Skretting Nutra Plus/Bio-Oregon Bio-vita Fry. Additional benefits in early survival and growth may be gained by supplementing prepared diets with live *Artemia* for Colorado R. cutthroat trout. The optimal temperatures for rearing cutthroat trout are subspecies-specific, ranging from 14.5°C and 14.7°C for Snake River and Yellowstone cutthroat trout, to 16.4°C for Colorado River cutthroat trout. Rearing density does have a slight but significant effect on growth, with fish reared at lower densities growing faster than those reared at higher densities. The production-scale trial demonstrated that the results of the laboratory studies could be used to predict growth rates at a production-scale.

**Impact:** The Colorado Division of Wildlife's Glenwood Springs Fish Hatchery switched to the premium diet used in the Colorado R. cutthroat trout study (like Skretting Nutra Plus/Bio-Oregon Bio-vita Fry) as a direct result of this study. The use of live *Artemia* supplementation during the first 21-d of production has not yet been adopted on a wide scale because of the added cost. However, a state hatchery in Alaska did express interest in using the technique to try to improve early survival of first-feeding grayling.

**Collaborators:** Colorado State University faculty in the College of Natural Resources, University of Idaho faculty in the Cooperative Extension Service, U.S. Fish and Wildlife Service personnel at the Bozeman Fish Technology Center, industry personnel at the Cline Trout Farms, Liley Fisheries, and Nelson and Sons, Inc..

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**PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:**

See attached sheets.

**SUBMITTED BY:**

	9/14/2010
Title: (Work Group Chair or PI)	Date

**APPROVED:**

	Date
Technical Advisor	Date

*Publications in Print*

- Brandt, M. M. 2009. Optimal starter diets and culture conditions for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). Colorado State University, Fort Collins, CO.
- Kindschi, G. A., and coauthors. 2009. Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture* 71(4):325-329.
- Brandt, M. M. 2008. Optimal culture conditions for first-feeding Colorado River cutthroat trout. *The Fishline* 20(2):1-16.

*Papers Presented*

- Myrick, C. A., M. Brandt, G. Kindschi and F. Barrows. 2009. Getting ahead in a cutthroat world: optimizing culture conditions for Snake River, Colorado River, and Yellowstone cutthroat trout. 60<sup>th</sup> Northwest Fish Culture Conference, Redding, CA.
- M. M. Brandt and C. A. Myrick. 2009. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Western Division of the American Fisheries Society, Albuquerque, NM.
- Myrick, C. A. 2009. Fish nutrition research at Colorado State University. Department of Animal Science Seminar, Fort Collins, CO.
- M. M. Brandt and C. A. Myrick. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society, Loveland, CO
- M. M. Brandt and C. A. Myrick. Optimizing survival and growth of first-feeding Colorado River cutthroat trout – diet temperature and density implications. Aquaculture America 2009, Seattle, WA.
- Brandt, M. M. and C. A. Myrick. 2008. Getting ahead in a cutthroat world: optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Idaho Aquaculture Association 2008 Annual Meeting, Twin Falls, ID, June 21-22.
- Brandt, M. M. and C. A. Myrick. 2008. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Colorado-Wyoming Chapter American Fisheries Society Meeting, Fort Collins, CO, March 3-6.
- Brandt, M. M. and C. A. Myrick. 2008. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Aquaculture America and World Aquaculture Society Joint Meeting, Orlando, FL, February 9-12.
- Brandt, M. M. and C. A. Myrick. 2008. Determination of optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Colorado Aquaculture Association 2008 Annual Meeting, Mt. Princeton, CO, January 18-19.
- Brandt, M. M. and C. A. Myrick. 2007. Getting ahead in a cutthroat world – performance of Colorado River cutthroat trout fed eight starter diets. 137<sup>th</sup> Annual Meeting of the American Fisheries Society, San Francisco, CA, September 2-6.
- Brandt, M. M. and C. A. Myrick. 2007. Getting a head start: growth of Colorado River cutthroat trout fed eight starter diets. Colorado-Wyoming Chapter American Fisheries Society Meeting, Fort Collins, CO, February 26-March 1.
- Brandt, M. M. and C. A. Myrick. 2007. Getting a head start: growth of Colorado River cutthroat trout fed eight diets. Colorado Aquaculture Association 2007 Annual Meeting, Mt. Princeton, CO, January 19-20.

- Fornshell, G. 2006. Aquaculture in the West. A WRAC Perspective (and other stuff too).  
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- Myrick, C. A. 2004. Development and evaluation of starter diets and culture conditions for 3  
subspecies of cutthroat trout and Gila trout: an introduction to the upcoming WRAC project.  
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11.