

THE EVALUATION OF PELLET TYPE-PREFERENCE AND DIETARY SOY
SENSITIVITY IN SNAKE RIVER CUTTHROAT TROUT
(*Oncorhynchus clarkia*)

by
Brian Roy Ham

A thesis submitted in partial fulfillment
of the requirements for the degree

of
Master of Science
In
Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2014

©COPYRIGHT

by

Brian Roy Ham

2014

All Rights Reserved

ACKNOWLEDGEMENTS

I would like to thank my advisors Dr. Wendy Sealey, Dr. Carl Yeoman, and Dr. Glenn Duff for helping me through this great opportunity. Through this process I was able to make contacts within the industry, see many different aspects of fish culture in many different environments, and travel the country attending very informational conferences broadening my knowledge. This was a great opportunity and again I would like to thank you for all of your support.

I would like to thank the US Fish and Wildlife Service staff, both the Bozeman Fish Technology Center and Creston National Fish Hatchery. The Bozeman Fish Technology staff including Matt Toner, Jason Illgen, Cal Fraser, Aaron Nistler, Eli Cureton, and Dr. Gibson Gaylord was very helpful in assisting me with fish culture, study breakdowns, and conducting proximate analysis in the analytical labs. The Creston National Fish Hatchery staff including Mark Maskill, Travis Slivka, Gar Holmes, and Sharon Hooley, provided support and career guidance through the SCEP/Pathways Intern program. I also would like to thank the USDA-ARS staff Dr. Rick Barrows and Jason Frost for their help in preparing diets, and providing a first class experience in the fish feed making process. I would like to thank the Western Regional Aquaculture Company for funding these projects. The cooperation and willingness of all these individuals was crucial to help me achieve this degree.

Finally I would like to thank my very understanding family for all of their support. Specifically, my wife Echo, my parents, and my sister for their unending support through it all.

TABLE OF CONTENTS

1. INTRODUCTION	1
Cutthroat Trout Background & Objectives	1
2. LITERATURE REVIEW	5
Cutthroat Trout	5
Salmonid Nutrition	9
Commercial Fish Feed Manufacturing: Extrusion	11
Plant-Based Protein Utilization in Commercial Trout Feeds	12
Soy Enteritis in Salmonids	12
3. EVALUATION OF THE EFFECTS OF PELLET TYPE ON GROWTH OF CUTTHROAT TROUT	15
Contributions of Authors and Co-Authors	15
Manuscript Information Page	16
Abstract	18
Introductions	19
Materials and Methods	21
Experimental Design	21
Experimental Subjects	22
Diet Formulation and Production	22
Westslope Cutthroat Feeding Trial	24
Snake River Cutthroat Feeding Trial	24
Fish Sampling	25
Fish Performance Indices Formulae	25
Proximate Composition Analyses	26
Statistical Analyses	26
Results	27
Westslope Feeding Trial Experiment 1	27
Snake River Cutthroat Trial Experiment 2	27
Discussion	29
Conclusion	32
Acknowledgement	32
References	40
4. THE EVALUATION OF DIETARY SOY SENSITIVITY IN SNAKE RIVER CUTTHROAT TROUT	43
Contributions of Authors and Co-Authors	43

TABLE OF CONTENTS - CONTINUED

Manuscript Information Page	44
Abstract	46
Introduction	47
Materials and Methods	49
Experimental Design.....	49
Experimental Subjects	50
Diet Formulation and Production.....	50
Feeding Trial.....	51
Fish Performance Indices Formulae	51
Sampling	51
Fish Condition Indices Formulae.....	52
Proximate Composition Analyses.....	52
Statistical Analyses	53
Results	53
Discussion	54
Conclusions	59
Acknowledgements	59
References	66
5. CONCLUSIONS	72
REFERENCES CITED.....	74

LIST OF TABLES

Table	Page
3.1	Composition of the experimental formulation fed to juvenile Westslope and Snake River cutthroat33
3.2	Analyzed diet composition of pellet types on an as-fed basis to Westslope cutthroat in study one and Snake River cutthroat in study two.....34
3.3	Growth of Westslope cutthroat juveniles ¹ fed varying pellet types in two different tank sizes.....35
3.4	Proximate composition of Westslope cutthroat juveniles ¹ fed varying diet types in two different tank sizes36
3.5	Growth of Snake River cutthroat juveniles ¹ fed varying pellet Types.....37
3.6	Body condition indices ¹ of Snake River cutthroat trout fed varying pellet types38
3.7	Whole body proximate composition ¹ of Snake River cutthroat trout fed varying pellet types39
4.1	Composition of the experimental formulation fed to juvenile Snake River cutthroat.....61
4.2	Growth of Snake River cutthroat juveniles ^a fed either soy protein concentrate or soy bean meal at 0, 5, 10, 15, or 30% for 10 weeks63
4.3	Body condition indices of Snake River cutthroat juveniles ^a fed either soy protein concentrate or soy bean meal at 0,5,10,15,or 30% for 10 weeks.....64
4.4	Proximate composition of Snake River cutthroat juveniles ^a fed either soy protein concentrate or soy bean meal at 0, 5, 10, 15, or 30% for 10 weeks65

ABSTRACT

Many sub-species of cutthroat trout are considered threatened or endangered in the United States. This has created an emphasis to transition from Rainbow trout to this lesser domesticated salmonid species; however, rearing cutthroat trout on commercially available rainbow trout diets has resulted in decreased performance in the hatchery. Research in other species has shown characteristics such as size, shape, color, and movement all affect interest in feed, while taste and texture influence consumption. A recent trend of decreasing fishmeal and increasing plant protein levels in salmonid diets has raised the question of how less domesticated species tolerate alternative ingredients. Therefore, the objectives of this study were to examine whether pellet-type preference affects the growth of juvenile cutthroat trout, and to examine how inclusion of soy-based protein sources affect the growth of this species, in hopes to develop a diet that improves the success of cutthroat trout hatchery culture. Two feeding trials were conducted. In the first, Westslope and Snake River cutthroat trout were fed a single experimental formulation manufactured to display four different pellet characteristics (floating, sinking, semi-moist, and flake). Consumption, weight gain and survival were compared. Results demonstrated a significant effect based on pellet type ($P=0.008$) but not stocking density ($P=0.0950$) on weight gain. Fish fed the flake grew significantly less than those fed other dietary treatments, with a 74% percent increase in weight gain ($P=.0001$). To address the effects of inclusion of dietary soybean meal and soy protein concentrate on growth efficiency, Snake River cutthroat trout were fed a practical-type formulation with 0, 5, 10, 15 or 30 % inclusion of dietary soybean meal or soy protein concentrate. Consumption, weight gain, nutrient retention and survival were compared. Final fish weight was altered by dietary soy inclusion level ($P=0.0001$) but not type ($P=0.779$). Snake River cutthroat fed the highest level of soy protein inclusion (30%) were significantly larger than fish fed other diets ($P=0.0001$). Additional research is needed to address the effects of soy on gut health and validate these results. Results from these studies can help hatchery managers choose appropriate feed types for culturing cutthroat trout in hatcheries.

CHAPTER ONE

INTRODUCTION

Cutthroat Trout Background & Objectives

Rainbow trout have been farmed in a hatchery environment for the better part of the century and through this time a great deal of information has been obtained to improve the aquaculture practices and thereby culture success of this species. Improved spawning techniques, genetic selection for faster growth, rearing techniques (Piper et al. 1984) and nutritional requirements (NRC 2011) have been thoroughly investigated. Such focus has been lacking in the development of other fish species and therefore culture of related species often follows Rainbow trout culture protocols (refer to Fish Hatchery Management Piper et al. 1984 for Rainbow trout protocols.)

Recently, increased emphasis has been placed on production of native fish species, as alternatives to Rainbow trout, particularly in areas where native trout species populations have decreased. One species of interest is the cutthroat trout, *Oncorhynchus clarkii*. Cutthroat are native to the western United States (Behnke 1992). There are several subspecies including coastal (*O. clarkii clarkii*), Westslope (*O. clarkii lewisi*), Yellowstone (*O. clarkii bouvieri*), and Lahontan (*O. clarkia henshawi*). All of these subspecies are related therefore gaining a better understanding of one of them may help improve the culture practices of the other species as well. A variety of inland cutthroat species including those mentioned above are currently being propagated in both public and private hatcheries to prevent the loss of the genetic purity and value of the resource

(Hilderbrand, 2002; Springer, 2004). As more facilities have attempted to culture these cutthroat, inconsistent (and often non-repeatable from year to year) growth, survival, and quality during the early life stages has been observed.

Preliminary research regarding cutthroat culture needs have attempted to reduce the problems of unpredictable growth performance. These studies have addressed thermal tolerance (Bear et al. 2007; Johnstone and Rahel 2003), optimum rearing density (Kindschi and Koby 1994; Wagner et al. 1997) and commercial diet suitability evaluations for broodstock, larvae, and fry (Arndt and Wagner 2007; Arndt et al. 1998; Edsall 1987; Kindschi et al. 2009; Myrick et al. 2010; Smith et al. 2004). These studies have demonstrated that although genetically similar, substantial differences exist between Rainbow trout and cutthroat trout culture requirements and feeding behavior.

Cutthroat trout are opportunistic feeders, however, fish smaller than 6 cm mostly consume plankton, whereas fish larger than 7 cm eat larger benthic prey (Luecke, 1986). Juveniles larger than 20 cm mostly consume fish and macro invertebrates, maximizing calorie intake per prey item (Nowak et al. 2004). Arndt et al. (1998) reported that when cutthroat trout were fed a floating diet with a demand feeder, growth and feed efficiency were improved compared to those fish fed a sinking diet fed by either hand or demand feeder. An important limitation of this work, however, was that by using commercial diets the authors' were unable to maintain equivalent nutrient composition, specifically in regards to lipid content. Therefore, the first objective of the current study was to examine if pellet-type preference altered performance of Snake River cutthroat trout when nutrient composition was equivalent.

Different salmonid species are known to utilize feeds with similar ingredient composition with varying efficiency (Azevedo et al. 2004). Even so, to date, cutthroat trout hatcheries continue to use commercial trout or salmon feeds with little or no fortification of nutrients. In the last two decades, commercial Rainbow trout diets have transitioned from what was historically a fishmeal protein-based feed to include larger amounts of a variety of plant-based protein sources. Soybean meal and soy protein concentrate, specifically, have been used because they are high in available protein, have a well balanced amino acid profile, and are reasonably priced compared to fish meal at this current time, and there is a steady supply of soy beans (Refstie et al. 2000). However, salmonids are known to be soy-sensitive with substantial variation between species and strain. Rainbow trout fed 50% inclusion of defatted soybean meal had reduced growth, feed efficiencies, and retention of nutrients (Rumsey et al. 1994). Atlantic salmon were able to tolerate 20% inclusion of defatted soybean meal, but when increased to 40% there was a decrease in growth (Olli et al. 1995; Refstie et al. 1998). Therefore, the second objective of this study was to examine Snake River cutthroat tolerance to soy-based protein sources at varying levels of inclusion.

These results would aid hatchery managers in the selection or modification of existing feeds to improve the performance of cutthroat trout in hatchery culture situations. The proper selection of a diet paired with the appropriate water temperature for rearing these fish will have great economic benefits for the growers. The Western Regional Aquatic Center research group reported the best growth with Snake River cutthroat trout raised on premium salmonid diets with almost 54% protein, and 18% lipid.

They also reported that supplementing or enriching diets with *Artemia* is a great way to improve survival from first feeding up to 21 days.

CHAPTER TWO

LITERATURE REVIEW

Cutthroat Trout

Cutthroat trout, *Oncorhynchus clarkii*, historically occupied a wide range of habitats, from small headwater streams, all the way to large rivers and everything in between throughout western Montana, Idaho, northwest Wyoming, eastern Oregon, and Washington (Linknes and Graham 1988; Behnke 1992; Thurow et al. 1997; Shepard et al. 2005). Due to vast changes throughout their historical range, the species has become very fragmented (Thurow et al. 1997) and due to the diminishing population, this species was recently reviewed to determine if it qualified to be listed as a threatened species under the Endangered Species Act (USFWS 2003). Although, the reviewers felt that at the time listing the species was not warranted, it still remains a species of concern for state and federal fisheries management agencies. The managers recognize that the species is at great risk due to habitat degradation, hybridization with nonnatives such as Rainbow trout (*Oncorhynchus mykiss*), and displacement or replacement by non-native species (Shepard et al. 1997, 2005; Shepard 2004; Rubidge and Taylor 2005). For these reasons, management agencies have instituted propagation and recovery programs for this species.

All inland cutthroat trout, are currently propagated in public hatcheries to prevent extirpation of these unique strains of fish (Hilderbrand 2002; Springer 2004). Currently Montana hatchery programs, at both the state and federal levels as well as private growers are attempting to rear this species both for conservation and recreational

purposes (Kindchi et al. 2009). Although there is both a demand and a need for hatchery reared cutthroat trout, growth, survival and quality during early life stages are inconsistent and non-repeatable from year to year (Hardy, 2001). There are several influences on this species that make it difficult to rear in the hatchery, including incomplete but growing knowledge of temperature tolerances (Smith et al. 1983), egg fertilization processes, transport requirements (Wagner et al. 2006), and proper oxygen levels (Kindschi and Koby 1994). As we have learned more about the previously mentioned topics, they have improved the efficiencies of raising cutthroat trout, but Wagner et al. (1997) and Fraley (2002) report that there are still unidentified problems resulting in fin erosion, increased disease incidence, decreased survival, and slow growth. Although likely multifaceted, nutritional deficiencies have been hypothesized, and with the help of an improved diet for this species perhaps some of these issues could be alleviated.

Rainbow trout have been farmed in a hatchery environment for the better part of the century (T. McMahon, MSU), and through this time a great deal of information has been obtained to improve the aquaculture practices and thereby culture success of this species. Spawning techniques, genetic selection for faster growth performance, rearing techniques (Piper et al. 1984), and diet quality (NRC, 2011) have been investigated. Such focus has been lacking in the development of other fish species and therefore culture of related species often follows Rainbow trout culture protocols (refer to Fish Hatchery Management Piper et al. 1984 for Rainbow trout protocols.)

In recent years, a growing number of studies on cutthroat trout have been completed that provide information relevant to rearing the species. These include studies on thermal tolerance (Bear et al. 2007; Johnstone and Rahel, 2003), rearing density (Kindschi and Koby 1994; Wagner et al. 1997) and diet development for broodstock, larvae, and fry (Arndt and Wagner 2007; Edsall 1987; Kindschi et al. 2009; Myrick et al. 2010; Smith et al. 2004). These studies have demonstrated that although related, substantial differences exist between Rainbow trout and Cutthroat trout. Kindschi and Koby (1994) demonstrated in Cutthroat trout that a fish density optimum was observed where increased survival, feed conversion, and weight gains were observed. The densities identified for having the best-feed conversions were 1555.8 (g)/ Liter and 4789.9 (g)/ Liter. Based on these results, current hatchery practice is to culture cutthroat trout at densities lower than those used for Rainbow trout.

Temperature is also a crucial aspect to raising this species in a hatchery environment. A study by Wagner et al. (1997) surveyed multiple hatcheries raising Rainbow Trout and then conducted a study using the Bonneville cutthroat trout and measured performance at two different temperatures, 13.4°C and 17.2°C. Growth was significantly greater with the warmer temperature although the fish did not convert the feed as effectively (Wagner et al. 1997). However, there is an upper limit as to how high the rearing temperature can be and increase growth. A decline in growth efficiency at higher temperatures in other salmonid species such as Atlantic salmon (Dwyer and Piper, 1987) and Brook trout (Dwyer et al. 1983). The upper lethal temperature of Westslope cutthroat trout was reported as 24.1°C (Bear et al. 2007). This is one of the lower upper

thermal limits of related salmonids (McCullough, 1999). The peak temperature for Rainbow trout feeding is between 15°C and 16°C (Alanara 1992, 1996). These differences in temperature tolerance may also explain why wild populations of Rainbow trout are outcompeting Cutthroat trout for resources given the increasing stream temperature trends over the last decade (Bear et al. 2007).

However, Cutthroat trout also have lower growth performance than Rainbow trout in hatchery settings (Mark Maskill USFWS). Inadequacy of commercially available Rainbow trout diets to support optimal growth of cutthroat in culture has been hypothesized as an explanation for this reduced performance. Historically, Rainbow trout diets contained high levels of protein and low levels of carbohydrate and lipid. Recently, there has been a shift in commercial diet formulations toward lower protein and higher fat formulations with more plant ingredient inclusion (NRC, 2011). These changes have implications for other species (like Cutthroat) fed these modern diets. Previous work has shown that from the first feeding of Cutthroat trout up to 120 days the best growth and survival was observed when the fish were fed a premium salmonid diet (Kindchi et al. 2009). Other related work from (Myrick et al. 2010) showed that the initial growth rates of Cutthroat trout fed premium salmonid diets in hatchery production systems were similar to laboratory conditions, but after the fish were transitioned to a regular trout production diet for grow-out to 20-30 cm total fish length (the desirable range for the recreational market) there was a decreased performance in growth compared to Rainbow trout at this life stage. This reduction in growth affects both product and

revenue loss, and could be alleviated by genetic selection, optimization of rearing techniques, and/or species-specific diet optimization.

Salmonid Nutrition

Cutthroat trout are opportunistic feeders and as they mature, their feeding characteristics change. Fish smaller than 6cm eating primarily plankton and fish larger than 7 cm moving from plankton to benthic prey (Luecke, 1986). Fish larger than 20 cm consuming mostly macro invertebrates, which allows them to maximize the amount of effort for corresponding caloric intake (Nowak et al 2004). Currently there is no species-specific diet available for cutthroat trout (Hardy 2001). This led growers to explore different options such as enriching current salmonid diets (Arndt and Wagner 2007), along with improving broodstock diets in hopes to increase the availability of trace minerals that would result in healthier brood fish and therefore better egg fertilization rates (Smith et al. 2004). Unfortunately, both of these techniques have had little success and are very inconsistent in reducing mortalities, increasing growth rates, and giving an overall more uniform rearing condition (Smith et al. 2004). Thus, a more stepwise approach to assessing the nutritional needs and dietary preferences of cutthroat trout could be beneficial.

Different salmonid species utilize feeds with similar ingredients with varying efficiencies and show preference for different characteristics such as floating vs. sinking feeds (Azevedo et al. 2004). Cutthroat trout are a relatively new species to the hatchery culture therefore much of what is currently known regarding feeding behavior and

preferences have been inferred from other salmonid species. Juvenile Atlantic salmon are said to be visual feeders, and when they are looking for prey, characteristics such as size, shape, color, and movement all play important rolls in making the fish commit; once the fish has committed to that piece of food then taste and texture influence the likelihood of its ingestion (Stradmeyer 1989). Stradmeyer et al. (1988) when examining what shape of pellet the fish preferred, reported that Atlantic salmon preferred longer thinner pellets over round fat ones. Texture has been reported to alter salmonid feed intake (Edsall et al. 1986). Edsall et al. 1986 examined the effect of various commercially available diets on growth of Cutthroat trout and demonstrated that fish fed a semi-moist feed supported the highest growth. However, a subsequent study by Kindschi et al. (2009) examining the effects of the same commercial brand semi-moist feed was not able to replicate the earlier results of Edsall et al. (1986). Previous work by Statler (1982), Kindschi (1984) and Tipping et al. (1986) reported better feed conversion rate of steelhead trout when fed using a demand feeder paired with sinking pellets. This was also true for Lake trout fed with a demand feeder (Aloisi 1994). These results have not always been observed with demand-fed Cutthroat trout (Wagner et al. 1995) and Rainbow trout (Wagner et al. 1996). In these latter studies, the hand-fed fish had better feed conversion rates. Automated feeders are often used when rearing cutthroat trout due to the lack of domestication of many Cutthroat subspecies and are hypothesized to reduce fish stress. Supplementation of feed by hand in conjunction with the use of feeders is being used at the Creston National Fish Hatchery with satisfactory success of survival and growth.

Commercial Fish Feed Manufacturing: Extrusion

The extrusion process was first used in the early 1970's for creating floating catfish diets (Arndt et al 1998), and through this process they noticed that an extruded pellet has some very beneficial characteristics that would work well for other species. The first was the ability to make the pellet float. This allowed the pellet to remain on the surface and allow ample time for the fish to see and then actively consume the pellet before it sank to the bottom of the tank. A second benefit of extrusion is that the pellet is much more stable making it less likely to leach nutrients if it were to remain uneaten at the bottom of the tank, ultimately polluting the tank (Arndt et al. 1998). The third advantage comes through dietary benefits. Extruded pellets are said to pass more slowly through the digestive system of Rainbow trout when compared to a steam-pelleted diets. Carbohydrate availability is also enhanced leading to increased gastric residence, which may ultimately increase the palatability of diets and ultimately increase feed conversions (Hilton et al. 1981). The extrusion process also cooks the ingredients at a very high temperature thus there is the possibility of nutrient degradation during the feed making process (Hilton et al. 1977; Lovell and Lim 1978). Specifically, in the cooking process vitamin C may be lost and the amino acids may also be destroyed (Hilton et al. 1981). To deal with these problems, fish feed manufacturers have been able to develop different ways of getting the proper amounts of vitamins and amino acids effectively through the extruder. For example, when using vitamin C, they have now moved to a product called StayC35. This product is able to handle the high temperatures without breaking down and still deliver the proper amounts of vitamin C to the fish.

Plant-Based Protein Utilization in Commercial Trout Feeds

The development of extrusion as the primary mode of commercial diet manufacture has also facilitated additional changes in commercial diet formulations. Historically, Rainbow trout diets contained high levels of protein from fishmeal and low levels of carbohydrate. Recently, there has been a shift in commercial diet formulations toward lower protein with more plant ingredient inclusion and higher fat formulations (NRC 2011). This shift is due primarily to the increasing price and limited availability of fishmeal coupled with the view of the utilization of plant protein sources as a sustainable alternative (Nordum et al. 2000). As increased utilization of plant protein meal has become more accepted as an alternative to fishmeal, soy products have been increasingly the most commonly used replacement (Nordum et al. 2000). The reason for the prominent use of soy products as a replacement is due to several reasons. Soybeans are much cheaper than fishmeal, a commodity that continues to rise. Second, soybeans are widely available (Nordum et al. 2000). Third, soybeans have a well balanced amino acid profile and a highly available protein content (Sealey et al. 2009).

Soy Enteritis In Salmonids

The types of soy products utilized in aquaculture are expanding. Currently the two most incorporated forms of soy are soybean meal and soy protein concentrate. Which differ substantially in their processing. Soybean meal is made by dehulling the soybean and then rolling the bean into a flake. When the bean is rolled, the oil is collected by extraction with a solvent. The rolled beans, which are termed flakes, are then toasted to

remove the trypsin inhibitor; this product is referred to as dehulled soybean meal that has about 48% crude protein. Along with the trypsin inhibitor that was removed with the toasting process there still remains several antinutritional compounds. These negative compounds generally reside in the carbohydrate fraction of the soybean meal, and include glycinin, B-conglycinin, oligosaccharides, lectins, and saponins (Liener, 1994). The removal of some antinutritional factors can be accomplished by further processing of the meal by treating it with different solvents to selectively remove the unwanted carbohydrates. The processes of treating, is most commonly aqueous alcohol extraction or isoelectric leaching. When soybean meal is processed using these steps, it greatly increases the protein content up to 90% and reduces the anti-nutritional factors (use of soybean meal in the diets of non salmonids). There are negative side effects associated with soy as listed previously and can be alleviated by further processing of soybean meal into products referred to as soy protein concentrate or soy protein isolate. When these more refined products are used, many of the anti-nutritional factors can be eliminated or reduced (Krogdahl et al., 1994; Buttle et al., 2001). The additional processing of soybean meal has a downside in that it greatly increases the cost of inclusion making it less cost effective when compared to fish meal (Sealey et al 2001).

Current inclusion rates of soybean meal in salmonid diets are generally kept at 20% or below to minimize negative effects on growth and health (Hardy 2002). When levels increase above 20%, there is a decrease in growth and decreased feed efficiencies (Olli and Krogdahl, 1995), along with other pathological changes such as inflammation in the distal intestinal epithelium causing diarrhea (Van den Ing et al. 1991 and Rumsey et

al. 1994). This change is referred to as enteritis (inflammation of the small intestine) (Baeverfjord and Krogdahl 1996 and Bakke-McKellup et al. 2000). Other issues include a decreased voluntary feed intake with soy diets, and subsequently reduced growth (Mambrini et al. 1999). Medale et al. 1998 reported that Rainbow trout feed intake was decreased when fish were fed a soy protein concentrate diet compared to a fish meal based diet. Hajen et al. (1993) reported that a soy protein concentrate (SPC) diet of only 15% inclusion displayed decreased palatability for Chinook salmon. While an issue for salmonid species, no reduction in growth or feed intake has been observed when soy ingredients replaced fishmeal in catfish diets (Webster et al., 1992; Kaushik et al., 1995). Salmonid species display substantial variation in their sensitivity with Rainbow trout being substantially more tolerant than Atlantic salmon (Refstie et al. 2000). To date, the sensitivity of Cutthroat trout, a lesser domesticated salmonid species, has not been examined but if present could, contribute to the decreased performance observed during hatchery culture.

CHAPTER THREE

EVALUATION OF THE EFFECTS OF PELLET TYPE ON GROWTH OF
CUTTHROAT TROUT

Contributions of Authors and Co-Authors

Manuscript in Chapter 3

Author: Brian R. Ham

Contributions: Organization of on site study, data collection, and preparing data.

Co-Author: Chris A. Myrick

Contributions: Study design and data interpretation

Co-Author: Frederic T. Barrows

Contributions: Feed formulation consulting

Co-Author: Carl Yeoman

Contributions: Study design and data interpretation

Co-Author: Glenn Duff

Contributions: Study design and data interpretation

Co-Author: Mark Maskill

Contributions: Study design and data interpretation

Co-Author: Wendy M. Sealey

Contributions: Study design, data interpretation, and guidance at the Bozeman Fish
Technology Center

Manuscript Information Page

Brian R. Ham, Chris A. Myrick, Frederic T. Barrows, Carl Yeoman, Glenn Duff, Mark Maskill, Wendy M. Sealey.

North American Journal of Aquaculture

Status of Manuscript:

Prepared for submission to a peer-reviewed journal

Officially submitted to a peer-review journal

Accepted by a peer-reviewed journal

Published in a peer-reviewed journal

American Fisheries Society

Evaluation of the Effects of Pellet Type on Growth of Cutthroat Trout

Brian R. Ham^{1,2,3}, Chris A. Myrick⁴, Frederic T. Barrows⁵, Carl Yeoman³, Glenn Duff³,
Mark Maskill² and Wendy M. Sealey¹

¹ USFWS, Bozeman Fish Technology Center, Bozeman, MT 59715, USA

² USFWS, Creston National Fish Hatchery, Creston, MT 59901, USA

³ Montana State University, Department of Animal and Range Sciences, Bozeman, MT
59901, USA

⁴ Colorado State University, Department of Fish, Wildlife, and Conservation Biology,
Fort Collins, CO 80523-1474, USA

⁵ USDA-ARS, Trout Grains Project, Hagerman Fish Culture Experiment Station, 3059F
National Fish Hatchery Road, Hagerman, ID 83332, USA

*Corresponding author's present address: Wendy M. Sealey, USFWS, Bozeman Fish
Technology Center, Bozeman, MT 59715, USA Wendy_Sealey@fws.gov

Abstract

Many sub-species of cutthroat trout are considered threatened or endangered in the North Western United States. This increases the necessity for Federal hatcheries to transition from rainbow trout to this lesser domesticated salmonid species. However, rearing cutthroat trout on commercially available rainbow trout diets has often resulted in decreased performance in the hatchery. Research in other species has shown characteristics such as size, shape, color, and movement all are important to making a fish ingest a piece of food. Taste and texture influence the likelihood of its retention. Therefore, the objective of this study was to examine whether pellet-type preference may help explain the decreased hatchery performance of juvenile cutthroat trout. To achieve this, we conducted two feeding trials where juvenile Westslope and Snake River cutthroat trout were fed a single experimental formulation manufactured to display four different pellet characteristics (floating, sinking, semi-moist, or flake) and compared consumption, weight gain and survival. In the first feeding trial, juvenile Westslope cutthroat (initial weight $11.3\text{g} \pm .5\text{ g}$) were stocked at a density of 20 fish per tank. Two different sizes of tanks were used with four replicate tanks for small tanks (54 L) and two replicate large tanks (96 L) per diet. In the second feeding trial, juvenile Snake River cutthroat trout (initial weight $19.5 \pm 0.5\text{ g}$) were stocked at 20 fish/tank in 96 L tanks with four replicate tanks per diet. Results of the first trial demonstrated a significant effect of pellet type ($P=0.008$) but not stocking density ($P=0.095$) on weight gain of Westslope cutthroat with no significant interaction ($P=0.584$). Westslope trout fed the flake diet grew significantly less than cutthroat fed all other dietary treatments. Feed conversion ratio (FCR) was

significantly affected by both pellet type ($P=0.0030$) and tank size ($P=0.0320$), with no significant interaction ($P=0.3835$). Results of the second trial also demonstrated that pellet type significantly affected growth efficiency of juvenile Snake River cutthroat trout ($P<0.001$). Fish fed the experimental formulation as a flake grew significantly less ($P=0.0001$), had higher FCR ($P=0.0001$), elevated HSI ($P=0.0003$), reduced muscle ratio ($P=0.0001$) and reduced more than all other treatments with only a 74% increase in weight gain ($P=0.0001$). Additional research is needed to address other culture related limitations. However, pellet-type preference observations from this study can help hatchery managers choose appropriate feed types for culturing cutthroat trout in hatcheries.

Introduction

Rainbow trout have been farmed in a hatchery environment for the better part of the century through this time, a great deal of information has been obtained to improve the aquaculture practices, and therefore culture success of this species. Improved spawning techniques, genetic selection for faster growth, rearing techniques (Piper et al. 1984), and nutritional requirements (NRC 2011) have been thoroughly investigated. Such focus has been lacking in the development of other fish species, and therefore culture of related species often follow rainbow trout culture protocols (refer to Fish Hatchery Management Piper et al. 1984 for rainbow trout protocols).

Recently, increased emphasis has been placed on production of native fish species as alternatives to rainbow trout, particularly in areas where native trout species

populations have decreased. One species of interest is the cutthroat trout, *Oncorhynchus clarkii*. Cutthroat trout are native to the western United States (Behnke 1992). There are several subspecies including coastal (*O. clarkii clarkii*), Westslope (*O. clarkii lewisi*), Yellowstone (*O. clarkii bouvieri*), and Lahontan (*O. clarkia henshawi*). A variety of inland cutthroat species, including those mentioned above, are currently being propagated in both public and private hatcheries to prevent the loss of the genetic purity and value of the resource (Hilderbrand, 2002; Springer, 2004). As more facilities have attempted to culture cutthroat, inconsistent and often non-repeatable from year to year growth, survival, and quality during the early life stages has been observed.

Preliminary research regarding cutthroat culture has attempted to reduce the problems of unpredictable growth performance. These studies have addressed thermal tolerance (Bear et al. 2007; Johnstone and Rahel 2003), suitable rearing density (Kindschi and Koby 1994; Wagner et al. 1997) and commercial diet suitability evaluations for broodstock, larvae, and fry (Arndt and Wagner 2007; Arndt et al. 1998; Edsall 1987; Kindschi et al. 2009; Myrick et al. 2010; Smith et al. 2004). These studies have demonstrated that although genetically similar, substantial differences exist between rainbow trout and cutthroat trout culture requirements and feeding behavior.

Research in other fish species indicates that characteristics such as size, shape, color, and movement all play important rolls in making a fish ingest a piece of food. Taste and texture influence the likelihood of its retention (Stradmeyer 1989). Cutthroat trout are opportunistic feeders, however, fish smaller than 6 cm mostly consume plankton, whereas fish larger than 7 cm eat larger benthic prey (Luecke 1986). Juveniles

larger than 20 cm mostly consume fish and macroinvertebrates, maximizing calorie intake per prey item (Nowak et al. 2004). Arndt et al. (1998) reported that when cutthroat trout were fed a floating diet with a demand feeder, growth and feed efficiency were improved compared to those fish fed a sinking diet fed by either hand or demand feeder. An important limitation of this work, however, was that by using commercial diets the authors were unable to maintain equivalent nutrient composition, specifically in regards to lipid content.

Different salmonid species are known to utilize feeds with similar ingredient composition with varying efficiency (Azevedo et al. 2004). Even so, to date, cutthroat trout hatcheries continue to use commercial trout or salmon feeds with little or no fortification of nutrients. Therefore, the objective of the current study was to examine the pellet type preference of Westslope and Snake River cutthroat trout when nutrient composition was equivalent. These results would thereby aid hatchery managers in the selection or modification of existing feeds to improve the performance of cutthroat trout in hatchery culture situations.

Materials and Methods

Experimental Design

Two 9-week feeding trials were conducted to examine the effect of pellet preference on growth of cutthroat trout. In the first trial, a 4 by 2 factorial design was employed where four different diet types (floating, sinking or semi-moist pellets or flakes) were fed to juvenile Westslope cutthroat trout cultured in either large (96 L) or

small (54 L) tanks. Fish were stocked 20 fish/tank and the diets were applied as a randomized block design where each diet was fed to two replicate large tanks of fish and five replicate small tanks of fish. In the second trial, juvenile Snake River cutthroat were randomly assigned to 20, 96 L tanks with four replicate tanks per diet and fed one of five different diet types (the floating, sinking or semi-moist pellets, flake, or the control) as compared to a commercial control diet.

Experimental Subjects

Juvenile Westslope cutthroat trout (approximately 7g) were obtained from Montana Department of Fish, Wildlife and Parks' Washoe Park Trout Hatchery, Anaconda, Montana and transported to the USFWS, Bozeman Fish Technology Center. Fish housed in this facility are of the MO12 strain. The MO12 strain was developed in 1983 and 1984 from populations in the Flathead and Clark Fork river drainages. Fish were stocked into 200 L tanks and maintained until start of the feeding trial. Snake River cutthroat were obtained as embryos from the Crystal River Colorado State Hatchery, Carbondale, CO and cultured in 200 L tanks until the start of the second feeding trial. Both feeding trials were conducted at the Bozeman Fish Technology Center, Bozeman, MT. All fish were handled and treated in accordance with the guidelines approved by the US Fish and Wildlife Service.

Diet Formulation and Production

To investigate pellet type preference, a diet (Table 3.1) with nutritional content that exceeded the NRC (2011) nutritional requirements for rainbow trout was formulated

to ensure that palatability was not limiting. The formulation was then processed in four different ways to form the feed. The processes included: cooking extrusion to yield a floating or sinking pellet, cold pelleting to produce a semi-moist pellet, and double drum drying to produce a flake feed.

Prior to pellet forming, all ingredients (Table 3.1) were ground using an air swept pulverizer. The floating and sinking pellets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with floating or sinking altered by method of oil inclusion; for floating pellets, oil was included post extrusion via top coat and for sinking pellets, oils were included prior to extrusion. Both diets had an 18-s exposure at an average of 127°C in the sixth extruder barrel section. The die plate was water cooled to an average temperature of 60°C. Pressure at the die head varied from 200 to 400 psi. Both cooking extruded and cold-pelleted diets were dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102°C with a 10-minute cooling period so that final moisture levels were less than 10%. Finally, the dried and cooled diets were top-coated with fish oil using a vacuum coater (A.J. Mixing, Ontario, Canada). The semi-moist pellets were produced by addition of glycerin and GDL to the basal diet and then cold pelleted (DNDL-44, Buhler AG, Uzwil, Switzerland). Soft moist pellets were air dried to approximately 30% moisture and stored frozen. All pellets were measured at approximately 2 mm finished diameter. The flake diet was formed using a double drum drier (30 x 46 cm drums) operated at atmospheric pressure supplied with steam (207 kN/m²), and run at a speed of 550 rotations/min to provide a 9s exposure to the drum surface (Caldwell et al. 2009).

Westslope Cutthroat Feeding Trial

Westslope cutthroat were randomly selected, group weighed and stocked 20 fish per tank into eight, 96 L and twenty, 54 L circular fiberglass tanks, with two replicate large tanks and five replicate small tanks of fish per diet. Spring water flow was set at 7 L/minute to maintain a constant temperature of 14°C and provide approximately four water exchanges per hour in large tanks and eight water exchanges per hour in small tanks and maintain an approximate dissolved oxygen level of 9.5mg/L. Hatchery raised Westslope cutthroat have been reported to grow optimally between 13°C and 15°C (Bear et al. 2007). Fish were fed daily using an automatic belt feeder that supplied a constant feed ration over a 12-h period from about 0800 to 2000 hours. Fish were fed amounts in excess of satiation (4% BW/d), as indicated by the presence of excess feed in tanks after a 12-h feeding cycle. Tanks were cleaned daily and mortalities removed and weighed. Temperature and dissolved oxygen concentration were measured daily. Photoperiod over the duration of the experiments was 13-h light and 11-h dark diurnal cycle.

Snake River Cutthroat Feeding Trial

Snake River cutthroat were randomly selected, group weighed and stocked 20 fish per tank into twenty, 96 L circular fiberglass tanks with four replicate tanks of fish per diet. Fish were fed by hand twice daily to apparent satiation. Water temperature was maintained at 14°C and lighting was maintained on a 13-h light and 11-h dark diurnal cycle. Fish were on a partial reuse system held at a constant 14°C with a flow of 7 L /minute yielding approximately 2 water exchanges per hour. This maintained the desired dissolved oxygen level of 9.5 mg/L. The commercial pellet chosen for comparison in this

study was a commercial slow sinking 2 mm diet (slow sinking, Skretting North America, Toole, Utah). This diet was chosen as a control because Creston National Fish Hatchery uses this feed for cutthroat this size.

Fish Sampling

At the start of the feeding trials, five fish from the source population were randomly selected, pooled, and frozen for determination of whole body composition. To monitor growth, all fish in both studies were counted and group weighed every three weeks. Feed intake was monitored weekly. At the conclusion of both feeding trials, five fish per tank were euthanized with a lethal dose of triacaine methane sulfonate (200 mg/L) for determination of whole body composition. At the end of the Snake River cutthroat feeding trial, five additional fish from each tank were randomly selected and dissected to determine visceral somatic index, hepatosomatic index, and muscle ratio.

Fish Performance Indices Formulae

Weight gain % increase = (average final wt. g/fish – initial wt. g/fish)/initial wt. g/fish x 100

Feed conversion ratio (FCR) = feed intake (dry weight)/body weight gain (wet weight)

Muscle ratio = fillet weight (g) X 2/ body weight (g) x 100

Hepatosomatic index = liver weight (g)/ body weight (g) x 100

Visceral index = viscera weight (g)/ body weight (g) x 100

Proximate Composition Analyses

Whole body fish samples were pooled by tank, ground for homogeneity, and frozen at -20°C until determination. Fish and diet samples were dried and analyzed in duplicate assays using standard AOAC (1995) methods for proximate composition. Protein was calculated from sample nitrogen content determined using a LECO TRUSPEC nitrogen analyzer (TruspecN, Leco Corporation, St. Joseph, Michigan, USA) and lipid using a Foss Tecator Soxtec HT Solvent Extractor, (Model Soxtec HT6 Höganäs, Sweden). Gross energy was determined by isoperibol bomb calorimetry (Parr 1281, Parr Instrument Company Inc., Moline, Illinois, USA).

Statistical Analyses

The PROC MIXED procedure, SAS Software Version 7.00 (SAS Institute, Inc., Cary, North Carolina) was used to conduct a factorial analysis of variance for a mixed effects model (Ott, 1977) in which pellet type and tank size were defined as fixed effects and tanks within treatments were defined as a random effect for the Westslope trial. For the Snake River trial, an analysis of variance for a mixed effects model was used. For all analyses, binomial data were transformed using the arcsine transformation prior to analysis. Differences within main effects were determined using the Tukey procedure for pair-wise comparisons (Tukey 1953). Treatment effects were considered different when probabilities were less than or equal to 0.05.

Results

Westslope Feeding Trial Experiment 1

No significant interactions between diet type and tank size were observed for juvenile Westslope cutthroat trout in the current study (Table 3.3). Survival in all treatments was high and ranged from 97% to 99% with no significant effect of treatment. In contrast, a significant effect of diet type ($P=0.008$) but not tank size ($P=0.095$) on weight gain of Westslope cutthroat with no significant interaction ($P=0.584$) was observed (Table 3.3). Westslope trout fed the flake diet grew significantly less than cutthroat fed all other dietary treatments. Feed conversion ratio (FCR) was significantly affected by both diet type ($P=0.003$) and tank size ($P=0.032$) with no significant interaction ($P=0.384$). Cutthroat trout fed the sinking diet had significantly lower FCR than fish fed either the semi-moist or flake feeds. Fish fed the flake diet had significantly higher FCR than all other diets. Tank size significantly altered FCR ($P=0.001$). Fish cultured in the large tanks had significantly higher FCR than fish in the small tanks.

Proximate composition was significantly altered by diet type ($P=0.018$) but not by tank size and no significant interaction was observed (Table 4). Westslope cutthroat trout consuming the flake diet has significantly lower whole body lipid levels compared to fish fed all other diet types.

Snake River Cutthroat Feeding Trial Experiment 2

Diet type significantly altered the growth of juvenile Snake River cutthroat trout ($P<0.001$; Table 5). Snake River cutthroat fed the experimental formulation as a semi-

moist, sinking, floating, control, or flake exhibited 269, 232, 231, 208, and 74 % increases in weight gain, respectively. The gains for fish fed the semi-moist, sinking or floating diets were significantly higher than those fed the commercial control diet (208%) and gains for fish fed the flake diet were significantly lower than all other treatments (74%). Feed conversion ratio was significantly higher in fish fed flake diets than all other treatments ($P < 0.0001$). Feed intake was significantly altered by pellet type with the highest intake observed in fish fed the flake, control and semi-moist diet, the feed intake was observed in fish fed the sinking pellet ($P = 0.005$). Survival was significantly reduced ($P < 0.001$) in fish fed the flake diet and Feed Conversion Ratios (FCR) was significantly increased in Snake River cutthroat trout fed the flake diet.

Body condition indices were also altered by diet type (Table 3.6). The visceral somatic index (VSI) of cutthroat fed the commercial diet was significantly higher ($P < 0.003$) than that of fish fed all other treatments. Hepatic Somatic Index (HSI) was significantly decreased ($P < 0.0003$) in fish fed the semi-moist diet type. Muscle ratios also were significantly ($P < 0.0001$) altered by diet type; muscle ratios of trout fed the floating, sinking, and semi-moist diets were significantly higher than fish fed the commercial control that was significantly higher than fish fed the flake diet.

Proximate composition was also significantly altered by diet type (Table 3.7). Fish fed the soft moist diet had the highest whole body lipid ($P = 0.0014$) and lowest whole body moisture (0.0103) while fish fed the flake diet had the lowest whole body energy ($P = 0.0059$). Whole body protein was not significantly altered by pellet type in Snake River cutthroat trout ($P = 0.7748$).

Discussion

Preliminary research regarding cutthroat culture needs has demonstrated that although genetically similar, substantial differences exist between rainbow trout and cutthroat trout diet preferences and feeding behavior. Experiments by Kindschi and Koby (1994) showed that high stocking density negatively affected the growth performance of Bar BC strain Snake River cutthroat trout. However, the densities used in their experiments were much greater than those used in the current studies. Specifically, Kindschi and Koby (1994) observed a negative effect at a density 4 times that of the recommended culture density (Piper, 1984). For comparison, in their most dense tank, the density was 247 g/L while in our Westslope experiment our most dense tank was 8 g/L and in our Snake River cutthroat study our highest density was 7 g/L. The authors did however, also report better growth correlating with increasing density up to a certain point and proposed that cutthroat trout at his lowest density 55 g/L may not have grown well due to the stress induced by such a small number of fish sharing a large amount of tank space (Kindschi and Koby 1994). Thus, the low growth rates observed for Westslope cutthroat in the current study could be explained by the low stocking density. However, in the Westslope experiment, the larger less dense tanks had better FCR's than that of the smaller more dense tanks making this seem unlikely. Additionally, similar densities did not suppress growth of the Snake River cutthroat in the current study. Snake River cutthroat stocked at approximately 7 g/ L demonstrated over 207% increase in growth in all treatments with the exception of the flake, increasing 77% during the 10 week study. These differences between growth rates when cultured in similar systems

likely indicate differences in culture stress tolerance between the subspecies, or possibly that these two strains of very closely related salmonids utilize the feeds of same composition differently. This idea is proposed by (Berg and Bremset 1998; Rasmussen and Ostefeld 2000; Refstie et al. 2000).

Cutthroat trout growth, survival, and quality during early life stages is inconsistent and not repeatable from year to year and diet insufficiency due to feed acceptance has been proposed at least, in part, as an explanation (Hardy 2001). Research in other fish species indicates that characteristics such as size, shape, color, and movement all play important rolls in making a fish ingest a piece of food, then taste and texture influence the likelihood of its retention (Stradmeyer, 1989). Previous work with cutthroat trout indicate that these factors likely influence cutthroat feed consumption in a hatchery setting. Edsal (1987) reported the best weight gain in Snake River cutthroat trout fed a commercial soft moist diet (Rangen Buhl, ID), while when Kindschi et al (2009) examined juvenile Snake River trout fed five commercial diets and two formulated diets, fish fed Skretting Nutra plus outperformed fish fed Rangen's soft-moist. Wagner et al. (1997) reported that fish fed with a floating diet by demand feeder had better feed conversion rates than compared to fish fed a sinking diet by hand. Arndt et al. (1998) reported that when cutthroat trout were fed a floating diet with a demand feeder, growth and feed efficiency were improved compared to those fish fed a sinking diet fed by either hand or demand feeder. An important limitation of these studies, however, was that by using commercial diets the authors were unable to maintain equivalent nutrient

composition, specifically in regards to lipid content thus in the present study, one formulation was manufactured to be nutritionally equivalent regardless of diet form.

In the current trials one formulation and a belt feeder was used to feed at 4% body weight for the Westslope trial while feeding by hand to satiation was used for the Snake River trial. At 4% body weight, the Westslope were fed to excess to ensure enough food was available for consumption over the 12-hour feeding period. The elevated FCR's in the Westslope study are likely due to feeding in excess to ensure adequate amounts of food were given to the fish. Belt feeders were used in the Westslope cutthroat study to overcome hand-feeding associated lack of consumption thought to be associated with the skittish behavior observed in this strain of cutthroat trout. Kindschi et al. (2009) fed Snake River cutthroat via belt feeders at 4% body weight and using this method they observed the highest growth in fish fed the Skretting Nutra-plus diet. The Skretting Nutra-plus diet is described by the company as a premium fry feed formulated with high-quality raw ingredients to ensure the best performance, and to have a slowing sinking characteristic to maximize feeding opportunities. In the current Snake River pellet study, fish were fed twice per day by hand to apparent satiation to more accurately measure the effect of feed type on intake. Intake rates of Snake River cutthroat in the current trials ranged from 2.3% to 2.7% BW/day. Overfeeding also likely explains the higher FCR of the flake fed Snake River cutthroat as well due to wastage of the flake due to sinking.

Conclusions

Our results indicate that the Westslope cutthroat grew best when fed a sinking diet via a belt feeder at 4% body weight per day, and there were no effects of densities observed. Snake River cutthroat trout performed the best when fed the semi-moist diet fed to apparent satiation. Importantly, a flake diet was not adequate for supporting growth of cutthroat at this life stage for both subspecies. In contrast, only minor differences were observed between other pellet types.

Substantial differences in growth performance were observed between Snake River and Westslope cutthroat recapitulating hatchery growth observations. These results can aid hatchery managers in the selection or modification of existing feeds to improve the performance of cutthroat trout in hatchery culture situations.

Acknowledgements

The authors wish to thank Jason Frost, Mark Portman, Clete Deshaser, Carly Stone, and Nick Leonard for their assistance with diet manufacturing, Matt Toner, Jason Ilgen, for their assistance with fish culture and sampling and Thomas O'Neill, Aaron Nistler and Omolola Betiku for their assistance with laboratory analyses. Funding for the study was provided, in part, by the Western Regional Aquaculture Center through grant number 2012-38500-19657 from the US department of Agriculture National Institute of Food and Agriculture. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply

recommendation or endorsement by the authors, the U.S. Department of Agriculture or the U.S. Fish and Wildlife Service.

Table 3.1 Composition of the experimental formulation fed to juvenile Westslope and Snake River cutthroat.

Ingredients¹	% as-fed
Sardine fish meal	37.2
Poultry By-Product meal	26.6
Wheat flour	11.5
Corn Protein Concentrate	5.3
Krill Meal	5.3
Spirulina	3.2
Wheat gluten meal	3.2
Menhaden fish oil	5.0
Lecithin	3.0
Stay-C 35	0.2
Vitamin premix ARS 702 ²	1.0
TM ARS 640 ³	0.1
Monocalcium Phosphate	2.0
DL-Methionine	0.25
Lysine HCl	1.50
Threonine	0.25
Taurine	0.50
Grobiotic A	0.20
Astaxanthin	0.05

¹ Ingredient sources: List ingredient and then source ex fishmeal (skretting, toole, UT); Gavilon LLC,(Omaha, NE, USA), MGP Ingredients, Inc. (Atchison, KS, USA).

² Contributed per kg of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D₃, 2160 IU; vitamin E (as DL- α -tocopheryl-acetate), 1590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamin mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; vitamin B₁₂, 90 ug.

³ Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3; selenium, 0.4

Table 3.2 Analyzed diet composition of pellet types on an as-fed basis to Westslope cutthroat in study one and Snake River cutthroat in study two.

Study	Pellet Type	Moisture (%)	Crude Protein (%)	Lipid (%)	Gross Energy (cal/gram)
Westslope	Floating	1.9	51.8	14.2	5195
	Sinking	1.8	51.0	16.9	5244
	Soft Moist	22.7	37.5	12.5	4327
	Flake	4.8	49.7	15.8	5138
Snake River	Floating	3.6	50.9	12.7	4987
	Sinking	1.0	50.7	17.5	5253
	Soft Moist	19.3	44.4	13.0	4198
	Flake	6.0	47.4	14.7	4968
	Control	7.6	47.0	18.3	5125

Table 3.3 Growth of Westslope cutthroat juveniles¹ fed varying pellet types in two different tank sizes.

Tank Size	Pellet Type	Initial fish weight (g)	Final fish weight (g)	Average Tank Gain (g)	Growth increase (%)	FCR	Survival
Small	Floating	11.6	21.2 ^a	199.5 ^a	83 ^a	2.7 ^b	100
	Sinking	11.3	21.4 ^a	191.3 ^a	90 ^a	2.5 ^b	99
	Soft Moist	11.3	21.2 ^a	193.2 ^a	87 ^a	3.2 ^b	99
	Flake	11.4	18.4 ^b	131.0 ^b	62 ^b	4.2 ^a	97
Large	Floating	11.6	21.5 ^a	155.4 ^a	85 ^a	4.1 ^b	90
	Sinking	11.4	21.3 ^a	197.4 ^a	87 ^a	2.6 ^b	100
	Soft Moist	11.2	19.1 ^a	153.1 ^a	70 ^a	3.6 ^b	95
	Flake	11.4	17.1 ^b	114.6 ^b	50 ^b	4.8 ^a	100
Pooled SEM		0.24	0.85	17	6	0.37	2.4
Pr>F ²		0.9352	0.0255	0.0178	0.0036	0.0012	0.3487
Tank Size		0.9707	0.2493	0.1061	0.1399	0.0321	0.2320
Pellet Type		0.5624	0.0047	0.0036	0.0005	0.0003	0.8670
Tank*Pellet ³		0.9852	0.6097	0.7543	0.5731	0.3835	0.1397

¹Means of two tanks per diet for the large tanks and five tanks per diet for the small tanks.

²Probability associated with the F-statistic.

³A significant (P<0.05) main effect of pellet type without significant interaction allowed data to be pooled; thus pellet type followed by different superscript letters are significantly different when pooled as determined by Tukey's LSD.

Table 3.4 Proximate composition of Westslope cutthroat juveniles¹ fed varying diet types in two different tank sizes.

	Pellet	Protein	Lipid	Moisture	Gross Energy
Tank Size	Type	%	%	%	(cal/g)
Small	Floating	14.7	7.1	77.1	1614
	Sinking	14.5	8.0	74.4	1686
	Soft Moist	14.3	7.1	75.0	1575
	Flake	14.8	6.1	76.2	1509
Large	Floating	14.9	6.2	76.5	1472
	Sinking	14.3	8.2	75.9	1478
	Soft Moist	14.6	7.7	75.0	1579
	Flake	15.9	6.8	74.4	1498
Pooled SEM		0.54	0.48	0.73	59
Pr>F ²		0.8388	0.0856	0.4800	0.2402
Tank Size		0.4547	0.7757	0.8359	0.0855
Diet Type		0.6418	0.0184	0.6088	0.3277
Tank*Diet Type ³		0.7870	0.5271	0.2158	0.3817

¹Means of five fish pools from each of two tanks per diet for the large tanks and five tanks per diet for the small tanks.

²Probability associated with the F-statistic.

³A significant (P<0.05) main effect of pellet type without significant interaction allowed data to be pooled; thus pellet type followed by different superscript letters are significantly different when pooled as determined by Tukey's LSD.

Table 3.5 Growth of Snake River cutthroat juveniles¹ fed varying pellet types.

Pellet Type	Initial fish		Final fish		Growth increase (%)	FCR	Feed Intake (%bw/d)	Survival (%)
	weight (g)	weight (g)	weight (g)	weight (g)				
Floating	19.6	64.9 ^b	231 ^b	2.1 ^b	2.4 ^{bc}	98 ^a		
Sinking	19.6	65.2 ^b	232 ^b	2.5 ^b	2.3 ^c	99 ^a		
Soft Moist	20.1	74.0 ^a	269 ^a	1.9 ^b	2.5 ^{ab}	99 ^a		
Flake	20.1	35.0 ^c	74 ^c	3.9 ^a	2.7 ^a	78 ^b		
Control	20.4	62.3 ^b	208 ^b	1.9 ^b	2.6 ^a	99 ^a		
Pooled SEM	0.3	2.2	11	0.2	1.3	1.3		
Pr>F ²	0.5360	0.0001	0.0001	0.0001	0.0001	0.0001		

¹Means of four tanks per diet.

²Probability associated with the F-statistic. Data within columns followed by different letters are significantly different as determined by Tukey's LSD.

Table 3.6 Body condition indices¹ of Snake River cutthroat trout fed varying pellet types.

Pellet Type	VSI²	HSI³	MR⁴
Floating	10.5 ^b	1.3 ^a	53.4 ^a
Sinking	10.2 ^b	1.3 ^a	53.4 ^a
Soft Moist	10.1 ^b	1.1 ^b	53.2 ^a
Flake	10.0 ^b	1.4 ^a	48.0 ^c
Control	11.2 ^a	1.3 ^a	51.4 ^b
<i>Pooled SEM</i>	0.57	0.09	0.64
<i>Pr>F⁵</i>	0.0030	0.0003	<0.0001

¹Means of four tanks (three fish/tank) per diet.

²VSI= Viseral Somatic Index, visera weight/body weight x 100.

³HSI= Viseral Somatic Index, liver weight/body weight x 100.

⁴MR= Muscle ratio, fillet weight x 2/body weight x 100.

⁵ Probablity associated with the F-statistic. Data within columns followed by different superscript letters are significantly different as determined by Tukey's LSD.

Table 3.7 Whole body proximate composition¹ of Snake River cutthroat trout fed varying pellet types.

Pellet Type	Protein	Lipid	Moisture	Gross Energy
	%	%	%	(cal/g)
Floating	16.3	7.7 ^c	74.5 ^a	1738 ^{ab}
Sinking	16.7	8.7 ^{ab}	72.9 ^{bc}	1737 ^{ab}
Soft Moist	16.3	9.3 ^a	72.2 ^c	1675 ^{bc}
Flake	16.9	8.0 ^{bc}	73.0 ^{bc}	1594 ^c
Control	16.8	7.6 ^c	73.9 ^{ab}	1775 ^a
<i>Pooled SEM</i>	0.45	0.25	0.4	30.15
<i>Pr>F²</i>	0.7748	0.0014	0.0103	0.0059

¹ Means of five fish pools from each of four tanks per diet.

² Probability associated with the F-statistic. Data within columns followed by different superscript letters are significantly different as determined by Tukey's LSD.

References

- AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.
- Arndt, R. E., & Wagner, E. J. (2007). Enriched Artemia and probiotic diets improve survival of Colorado River cutthroat trout larvae and fry. *North American journal of aquaculture*, 69(2), 190-196.
- Arndt, R. E., Wagner, E. J., Routledge, M. D., & Bradwisch, Q. A. (1998). Hatchery performance and fish health of cutthroat and rainbow trout hand-fed or demand-fed a floating feed. *Journal of applied aquaculture*, 8(3), 41-51.
- Azevedo, P. A., Leeson, S., Cho, C. Y., & Bureau, D. P. (2004). Growth and feed utilization of large size rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) reared in freshwater: diet and species effects, and responses over time. *Aquaculture nutrition*, 10(6), 401-411.
- Bear, E. A., McMahon, T. E., & Zale, A. V. (2007). Comparative thermal requirements of westslope cutthroat trout and rainbow trout: implications for species interactions and development of thermal protection standards. *Transactions of the American Fisheries Society*, 136(4), 1113-1121.
- Behnke, R. J. (1992). Native trout of western North America. *American Fisheries Society monograph (USA)*. no. 6.
- Berg, O. K., & Bremset, G. (1998). Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology*, 52(6), 1272-1288.
- Caldwell, C. A., Barrows, F. T., Ulibarri, M., & Gould, W. R. (2010). Diet Optimization of Juvenile Rio Grande Silvery Minnow. *North American Journal of Aquaculture*, 72(1), 57-64.
- Cunniff, P. (1995). Official methods of analysis of AOAC International.
- Edsall, D. A. (1987). Evaluation of starter diets for Snake River cutthroat trout. *The Progressive Fish-Culturist*, 49(2), 93-95.
- Hardy, R. W. (2002). Rainbow trout, *Oncorhynchus mykiss*. *Nutrient requirements and feeding of finfish for aquaculture*, 184-202.

- Hilderbrand, R. H. (2002). Simulating supplementation strategies for restoring and maintaining stream resident cutthroat trout populations. *North American Journal of Fisheries Management*, 22(3), 879-887.
- Johnstone, H. C., & Rahel, F. J. (2003). Assessing temperature tolerance of Bonneville cutthroat trout based on constant and cycling thermal regimes. *Transactions of the American Fisheries Society*, 132(1), 92-99.
- Kindschi, G. A., Myrick, C. A., Barrows, F. T., Toner, M., Fraser, W. C., Ilgen, J., & Beck, L. (2009). Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture*, 71(4), 325-329.
- Kindschi, G. A., & Koby Jr, R. F. (1994). Performance and oxygen consumption of Snake River cutthroat trout reared at four densities with supplemental oxygen. *The Progressive Fish-Culturist*, 56(1), 13-18.
- Luecke, C. (1986). Ontogenetic changes in feeding habits of juvenile cutthroat trout. *Transactions of the American Fisheries Society*, 115(5), 703-710.
- Maskill, Brian. *Cutthroat Trout Interview* 15 march 2012. March . Personal interview. McMahon, Tom. "Ichthyology." Montana State Universtiy. Montana, Bozeman. 16 Feburary 2012. Lecture.
- Myrick, C. A. (2008). Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout. *Western Regional Aquaculture Center Annual Progress Report, Colorado State University, Fort Collins Colorado*.
- National Research Council (2011). *Nutrient Requirements of Fish*. Washington, D.C., USA, National Academy Press.
- Nowak, G. M., Tabor, R. A., Warner, E. J., Fresh, K. L., & Quinn, T. P. (2004). Ontogenetic shifts in habitat and diet of cutthroat trout in Lake Washington, Washington. *North American Journal of Fisheries Management*, 24(2), 624-635.
- Ott, L. 1977. *An introduction to statistical methods and data analysis*. Duxbery Press, North Scituate, Massachusetts, USA.
- Piper, R. G. (1986). *Fish hatchery management*. US Dept. of the Interior, Fish and Wildlife Service.

- Rasmussen, R. S., & Ostefeld, T. H. (2000). Effect of growth rate on quality traits and feed utilisation of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Aquaculture*, 184(3), 327-337.
- Refstie, S., Korsøen, Ø. J., Storebakken, T., Baeverfjord, G., Lein, I., & Roem, A. J. (2000). Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 190(1), 49-63.
- Smith, M. A., Hubert, W. A., & Barrows, F. T. (2004). Failure of a plant-and-krill-based diet to affect the performance of Yellowstone Cutthroat trout broodfish. *North American Journal of Aquaculture*, 66(1), 61-69.
- Springer, C. 2004. Native trout conservation in the U.S. West. *Hatchery International* 5(6):16–18.
- Stradmeyer, L. (1989). A behavioural method to test feeding responses of fish to pelleted diets. *Aquaculture*, 79(1), 303-310.
- Tukey, J. (1953). *The problem of multiple comparisons*, Princeton University, Princeton, New Jersey, USA.
- Wagner, E. J., Jeppsen, T., Arndt, R., Routledge, M. D., & Bradwisch, Q. (1997). Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin erosion, and general health and condition. *The Progressive Fish-Culturist*, 59(3), 173-187.

CHAPTER FOUR

EVALUATION OF THE SOY TOLERANCE OF SNAKE RIVER
CUTTHROAT TROUT

Contributions of Authors and Co-Authors

Manuscript in Chapter 4

Authors: Brian R. Ham

Contributions: Experimental setup, data collection, and proximate analysis.

Co-Author: Frederic T. Barrows

Contributions: Feed manufacturing assistance

Co-Author: Carl Yeoman

Contributions: Data interpretation

Co-Author: Glenn Duff

Contributions: Data interpretation

Co-Author: Mark Maskill

Contributions: Data Interpretation

Co-Author: Wendy M. Sealey

Contributions: Encouragement through the entire process with knowledge relating to experiment design and feed formulation.

Manuscript Information Page

Brian R. Ham, Chris A. Myrick, Frederic T. Barrows, Carl Yeoman, Glenn Duff, Mark Maskill, Wendy M. Sealey.

Status of Manuscript:

- Prepared for submission to a peer-reviewed journal
- Officially submitted to a peer-review journal
- Accepted by a peer-reviewed journal
- Published in a peer-reviewed journal

The Evaluation of Dietary Soy Sensitivity in Snake River Cutthroat Trout
(*Oncorhynchus clarkia*)

Brian R. Ham^{1,2,3}, Frederic T. Barrows⁴, Carl Yeoman³, Glenn Duff³, Mark Maskill² and
Wendy M. Sealey¹, Amberly Huntiger⁵

¹ USFWS, Bozeman Fish Technology Center, Bozeman, MT 59715, USA

² USFWS, Creston National Fish Hatchery, Creston, MT 59901, USA

³ Montana State University, Department of Animal and Range Sciences, Bozeman, MT
59901, USA

⁴ USDA-ARS, Trout Grains Project, Hagerman Fish Culture Experiment Station, 3059F
National Fish Hatchery Road, Hagerman, ID 83332, USA

⁵USFWS, Bozeman Fish Health Lab, Bozeman, MT 59725, USA

*Corresponding author's present address: Wendy M. Sealey, USFWS, Bozeman Fish
Technology Center, Bozeman, MT 59715, USA Wendy_Sealey@fws.gov

Abstract

The shift in commercial rainbow trout diet formulations toward lower protein and higher fat formulations with more plant ingredient inclusion (NRC 2011) and, specifically increased soy product inclusion, may have negative implications for less domesticated trout species fed these modern diets in conservation hatcheries. Therefore, the objective of this study was to examine the effects of inclusion of dietary soybean meal and soy protein concentrate on growth efficiency of cutthroat trout. To achieve our objective, we conducted a feeding trial where juvenile Snake River cutthroat trout were fed a practical type formulation with 0, 5, 10, 15, or 30 inclusion of dietary soybean meal or soy protein concentrate and compared consumption, weight gain, nutrient retention and survival. Juvenile Snake River cutthroat trout (initial weight 28.1g/fish +/- standard deviation of 1.0g) were stocked at 20 fish/tank in 200 L tanks with three replicate tanks per diet and fed their respective diets for 10 weeks. Final fish weight was altered by dietary soy inclusion level ($P=0.0001$) but not type ($P=0.779$) and no interaction was observed ($P=0.6019$). Snake River cutthroat fed the diets with the highest level of soy protein inclusion (30%) were significantly larger than fish fed all other diets with a final average fish weight of greater than 130 g ($P=0.0001$). The FCR's were statistically higher in fish fed diets with 0% and 5 % soy inclusion than fish fed the 30% and 10% inclusion diets ($P=0.0044$). In contrast, no statistically significant effect of soy inclusion levels ($P=0.0825$) on intake as measured by percent body weight per day was observed. Additional research is needed to address the effects of soy on gut health and verify these

results; however, soy-type and level observations from this study can help hatchery managers choose appropriate feed types for culturing cutthroat trout in hatcheries.

Introduction

Plant-based protein inclusion levels in modern salmonid diets are much higher than traditional inclusion levels as many plant-based proteins are becoming accepted as sustainable alternatives (Nordum et al. 2000, Gatlin et al. 2007). Soy products, specifically, have garnered considerable attention as cost effective fishmeal alternatives (Gatlin et al. 2007). This transition has not been without challenges as the switch from fishmeal to plant-based protein sources has often resulted in decreased growth and increased FCR's (Barrows et al. 2007; Gomes et al. 1995). One identified reason for the decrease in growth characteristics is the inability of the amino acid profile of plants to meet the needs of fish protein synthesis (Lansard et al. 2010). When soy protein concentrate diets are supplemented with amino acids, the growth of rainbow trout fed these diets is not different from rainbow trout fed fish meal based diets (Aksnes et al. 2007; Cheng et al. 2003; Gaylord and Barrows, 2009; Snyder et al 2012). Inclusion rates of soybean meal in salmonid diets are kept at 20% or less (Hardy, 2002); however, because even when amino acids are balanced, feeding soybean meal above these levels has been shown to be detrimental (Olli and Krogdahl, 1995). Further processing soybean meal into soy protein concentrate can eliminate the anti-nutritional effects for most soy-sensitive species (Krogdahl et al., 1994; Buttle et al., 2001). Soybean meal anti-nutritional effects are most notable in the hindgut of the fish, where the intestinal tract

becomes inflamed characterized by increased mucosal leukocyte accumulations, epithelial cell proliferation and finally diarrhea when the intestine becomes inflamed (Rumsey et al. 1994). Deleterious effects also have been observed in Atlantic salmon fed soybean meal, where adenocarcinomas evolving through progressive epithelial dysplasia were associated with increased intestinal inflammation similar to human colorectal cancer (Dale et al. 2009). Additionally, decreases in immunocompetency have been observed and are believed to be linked to crucial immunogenic role of the hindgut of teleost fish in helping keep the organism healthy (Ellis 1995). Importantly, substantial variability in soy sensitivity has been observed across species fish feeding guilds (NRC 2011) as well as between salmonid species with those species that have undergone less domestication displaying increased sensitivity (Medale et al. 1998 and Hajen et al. 1983).

Recently, increased emphasis has been placed on production of native salmonid species for both conservation and recreation purposes, particularly in areas where native trout species populations have decreased. One species of interest in this regard is the cutthroat trout, *Oncorhynchus clarkia*. Cutthroat trout have lower growth performance than rainbow trout in hatchery settings and inadequacy of commercially available rainbow trout diets to support optimal growth of cutthroat in culture has been hypothesized as an explanation for this reduced performance. Previous work has shown that from first feeding cutthroat trout up to 120 days, the best growth and survival was observed when the fish were fed a premium salmonid diet (Kindchi et al. 2009). Other related work from Myrick et al. (2010) showed that the initial growth rates of cutthroat trout fed premium salmonid diets in hatchery production systems were similar to

laboratory conditions, but after the fish were transitioned to a regular trout production diet there was decreased growth performance compared to rainbow trout at the same life stage. These studies suggest that although genetically similar, differences may exist between rainbow trout and cutthroat trout regarding the suitability of commercial diets containing plant-based protein sources to support growth and health. The previously described shift in commercial rainbow trout diet formulations toward lower protein and higher fat formulations with more plant ingredient inclusion (NRC 2011) and specifically increased soy product inclusion may thereby have negative implications for cutthroat trout fed these modern diets. Therefore, the objective of this study was to examine the effects of 0,5,10, 15 and 30 % inclusion of dietary soybean meal and soy protein concentrate on intake, survival, growth efficiency and intestinal health of Snake River cutthroat trout.

Materials and Methods

Experimental Design

A twelve-week feeding trial was conducted to determine the effects of dietary inclusion of either soy protein concentrate or soybean meal (0, 5, 10, 15, or 30%) on growth performance, nutrient retention and proximate composition of juvenile Snake River cutthroat trout in a 2 X 5 factorial design. All fish rearing and sampling protocols were approved by the USFWS, Bozeman Fish Technology Center Animal Care and Use Committee.

Experimental Subjects

Snake River cutthroat trout were obtained as embryos from Crystal Springs Hatchery, Carbondale, CO and cultured at the Bozeman Fish Technology Center until the experiment commenced. The fish were acclimated to the experimental tanks for one week prior to the beginning of the study.

Diet Formulation and Production

A practical-type diet was formulated to meet or exceed the nutrient requirement of juvenile rainbow trout (NRC 2011; Hardy 2002). The diet was formulated to contain approximately 48% crude protein and 15% crude lipid with fish meal and poultry byproduct meal as the primary protein sources (Table 4.1). Test diets were formulated by substituting 0, 5, 10, 15, or 30 % soy protein concentrate or soybean meal for both protein sources using rainbow trout digestible protein values. Diets were balanced for methionine, lysine, threonine using the ideal amino acid targets of 3.8, 1.3, and 2.1%, respectively and total phosphorus of 1.5% through the addition of monocalcium phosphate. Prior to mixing, all ingredients were ground using an air-swept pulverizer (Jacobsen 18H, Minneapolis Minnesota, USA). Dry ingredients were mixed in a horizontal paddle mixer, Marion Mixers, Marion, Iowa, USA, and a portion (~1/3) of the added oil was mixed into the dry ingredients along with the lecithin. The mash was then extruded through a 3.0mm die of a Buhler twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). Barrel temperature averaged 124 °C in sections 2–6, and die pressure was ~360 psi and the feed had a barrel residence time of approximately 18 s. The diets were dried in a pulse bed drier extruder (Buhler AG, Uzwil, Switzerland)

with air discharge temperature remaining below 104 °C, and final moisture content less than 8%. After the diets were dried, they were top-coated with the remaining oil at ambient pressures, and stored at room temperature (~18–23 °C).

Feeding Trial

At stocking, Snake River cutthroat trout (28.1 g/fish +/- 1.0 g) were counted into groups (20 fish) and placed into 200-L growth tanks. Ten treatments randomly allocated, with three replicate tanks of fish for each treatment, for a total of 30 experimental tanks. Each tank was supplied with constant temperature (14° C) water at a flow rate of 8-L per minute. Photoperiod was maintained at a constant 13 h light and 11 h dark with fluorescent lighting.

Fish Performance Indices Formulae

Weight gain percent increase, = (average final wt. g/fish – initial wt. g/fish)/initial wt. g/fish x 100

Feed conversion ratio (FCR) = feed intake (dry weight)/body weight gain (wet weight)

Sampling

At the start of the feeding trials, five fish from the source population were randomly selected, pooled and frozen for determination of whole body composition. To monitor intake during this twelve-week trial, the fish were fed twice a day, in the morning and afternoon to approximate satiation by hand, feeding the fish all they will consume in thirty minutes. To monitor growth, the fish were counted and group weighed

every three weeks, and bucket weights were recorded every three weeks. At the conclusion of the feeding trial, ten fish per tank were euthanized with a lethal dose of triacaine methane sulfonate (200 mg/L). Five fish were pooled and frozen for determination of whole body composition. The five remaining fish were used for determining individual body composition indices.

Fish condition indices were calculated using the following formulae:

$$\text{Fillet ratio} = \text{fillet weight (g)} \times 2 / \text{body weight (g)} \times 100$$

$$\text{Hepatosomatic index} = \text{liver weight (g)} / \text{body weight (g)} \times 100$$

$$\text{Visceral index} = \text{viscera weight (g)} / \text{body weight (g)} \times 100$$

Proximate Composition Analyses

Proximate composition fish samples ground for homogeneity, and frozen at -20°C until determination. Fish and diet samples were dried and analyzed in duplicate assays using standard AOAC (1995) methods for proximate composition. Protein was calculated from sample nitrogen content determined using a Leco Truspec nitrogen analyzer (TruspecN, Leco Corporation, St. Joseph, Michigan, USA) and lipid using a Foss Tecator Soxtec HT Solvent Extractor, Model Soxtec HT6 (Höganäs, Sweden). Gross energy was determined by isoperibol bomb calorimetry (Parr 1281, Parr Instrument Company Inc., Moline, Illinois, USA).

Statistical Analyses

The PROC GLM procedure, SAS Software Version 7.00 (SAS institute, Inc., Cary, North Carolina) was used to conduct a factorial analysis of variance for a mixed effects model (Ott, 1977) in which soy type and level were defined as fixed effects and tanks within treatments were defined as a random effect. For all analyses, binomial data were transformed using the arcsine transformation prior to analysis. Differences within main effects were determined using the Tukey procedure for pair-wise comparisons (Tukey1953). Treatment effects in all statistical analyses in this project were considered different when probabilities were less than 0.05.

Results

There were no significant effects of soy type or interactions between type of soybean protein source included in the diet and the level of inclusion on Snake River cutthroat growth efficiency (Table 4.2). However, soy levels significantly altered all parameters except for feed intake. Survival in all treatments was high ranging from 96% to 100%. Final fish weight was altered by dietary soy inclusion level ($P=0.0001$) but not type ($P=0.779$) and no interaction was observed ($P=0.6019$). Snake River cutthroat fed the diets with the highest level of soy protein inclusion (30%) were significantly larger than fish fed all other diets with a final average fish weight of greater than 130 g ($P=0.0001$). Similarly, fish fed the diets with the 30% inclusion of soybean meal and concentrate had the highest growth rates observed (370% increase) in this ten-week period ($P=0.0001$). In contrast, no statistically significant effect of soy inclusion levels

($P=0.0825$) on intake as measured by percent body weight per day was observed. The FCRs were statistically higher in fish fed diets with 0% and 5% soy inclusion than fish fed the 30% and 10% inclusion diets ($P=0.0044$).

No significant effects of soy type, soy level or interactions between type of soybean protein source included in the diet and the level of inclusion on Snake River cutthroat on visceral somatic index or the hepatosomatic index among treatments were observed (Table 4.3). In contrast, the muscle ratio (MR) data did show statistical differences among the diets when soy level was altered ($P=0.0222$). Fish fed diets with 5%, 10%, or 15% soy products had the highest muscle ratios followed by fish fed 0% soy products. Fish fed diets containing 30% soy product had lower MR than all other treatments.

No significant effects of soy type, soy level or interactions between types of soybean protein source included in the diet and the level of inclusion on Snake River cutthroat whole body proximate composition (Table 4.4). Whole body protein and lipid, ranged from 13.6%-19.6% and 8.3%-9.9%, respectively. Whole body moisture levels ranged from 72.2%- 73.8% and whole body energy values ranged from 1673-1815 cal/g.

Discussion

Rainbow trout fed high levels of de-fatted soybean meal had reduced growth, feed efficiency and retention of nutrients (Rumsey et al. 1995). Similarly, Atlantic salmon displayed reduced growth when fed de-fatted soybean meal (Olli et al. 1995; Refstie et al. 1998). Therefore, soy inclusion levels in salmonid diets are generally kept at 20% or

below to minimize negative effects on growth and health (Hardy 2002). When levels increase above 20%, there is a decrease in growth and decreased feed efficiencies depending on species (Olli and Krogdahl, 1995). Refstie et al. (2000) compared growth rates of Atlantic salmon to Rainbow trout fed both a fish meal diet and a 30% soybean inclusion diet and reported that fish fed the fish meal diet grew more than the soy diet in both species, with 44% more weight gain over 12 weeks. In the current study, no similar decreases in growth or feed efficiency were observed when rearing Snake River cutthroat trout with up to 30% inclusion of either soybean meal or soy protein concentrate. Reasons for these differences remain unclear, but could include variation in species sensitivity, quality of the proteins used or the differing dietary formulation criteria.

Salmonids are known to be soy-sensitive with substantial variation between species. When young Chinook and Coho salmon were fed full-fat soybean meal diets, growth and feed efficiency was greatly reduced in both species; however, a more drastic growth reduction was observed in Chinook than was observed in Coho (Fowler 1980). That study also showed that as soybean meal inclusion levels increased, fish performance was inversely affected, which the authors attributed to the decrease in palatability of the diets with higher levels of soy (Fowler 1980). When comparing the results of Fowler (1980) to other salmonids such as Rainbow trout and Atlantic salmon, the latter species appear to be more tolerant to increasing levels of soy inclusion (Bureau et al. 1998). The growth data from the current study suggests that Snake River cutthroat trout may be more tolerant to soy protein inclusion than related salmonids. Previous research by Olli and

Kroghdahl (1995) indicated that Atlantic salmon might be more sensitive to the alcohol soluble carbohydrate component of soybean meal compared to Rainbow trout.

Differing quality of ingredients used in plant protein replacement studies, and thus their anti-nutritional content, has been shown to drastically affect project outcomes. The soybean meal used in this study was obtained from Archer Daniels Midlands as a commodity grade product. The soy protein concentrate used was obtained from Solae and was a human food grade product Profine VF product. Trypsin inhibitor that is known to be present in soybean meal does not appear to be an issue when the meal is heated (Olli et al. 1989; Rumsey et al. 1993) and the alcohol soluble carbohydrate portion of the soybean is partially responsible for causing the high levels of variability within the product, leading to low nutritional value (Arnesen et al. 1989; Olli et al. 1994). Olli and Kroghdahl (1994) conducted a feeding trial comparing different types of soy diets with up to 56% protein replacement when feeding Rainbow Trout and Atlantic salmon. The authors found no differences in anti-nutritional factors among treatments, though there was decreased growth performance noted with increase levels of replacement. Although antinutritional factors were not measured in the present study, the increase in Snake River trout growth performance observed with increasing levels of soy protein thus could indicate these factors either were not present or were present in low amounts.

Quality of protein ingredients in terms of their ability to meet the essential amino acids and mineral needs of salmonids can also alter success of soy replacement study project outcomes. When replacing fishmeal with soy products, the fish becomes limited and the 10 essential amino acids requirements are not met (Hardy, 2002), especially

methionine, threonine, and lysine. Other deficiencies occur with decreasing amounts of available phosphorus and minerals. It has been shown that supplementing limiting amino acids improves growth in salmonid species when replacing fish meal with plant-based diets (Davies and Morris, 1997 and Yamamoto et al. 2005). In the current study, an ideal amino acid concept was used (Gaylord and Rawles, 2005). The diets in this study were formulated to exceed the requirements of rainbow trout which for protein requirements is defined as 42-48% (Hardy, 2002). Our diets contained 49-51% protein, so we exceeded the requirements as stated for rainbow trout, so when comparing the performance of our control diet to the higher levels of soy inclusion, we supplemented more amino acids which have been shown to help improve protein retention (Rodehutscord et al. 2000), and we maintained a very high level of percent protein in the diet of 50%. This high level of protein with additional supplementation of amino acids could, in part, explain the increased growth observed with the higher levels of soy inclusion treatments.

Other issues observed when soy products are included in fish diets are decreased voluntary feed intake with soy-containing diets (Hajen et al. 1993; Mambrini et al. 1999; Medale et al. 1998). Bureau et al. (1998) showed a decrease in growth when feeding high levels of soybean meal diets with high levels of measured soyaaponins compared to a soy protein isolate diet. The decrease in growth was attributed to a significant finding of decrease in feed intake. Although, we did not measure our soyaaponin levels in the current study, no significant difference in feed intake due to level of inclusion or type of soy protein inclusion.

Studies have shown with an increase in rainbow trout dietary soy level, decreased apparent lipid and energy digestibility are often observed (Pngmaneerat and Wantabe 1993). Effects on lipid and energy digestibility were also greater when soybean meal was used when compared to soy protein concentrates. This was demonstrated in a study using Atlantic salmon, where the use of soybean meal led to higher fecal concentrations of lipid, but at the same concentration of soy protein concentrate, no difference of fecal lipid was noted (Olli et al., 1995). The physical properties of soy may affect lipid digestibility in several ways. Soy proteins are amphiphilic globulins; these absorb fats and the indigestible part of soy products (Lusas and Riaz 1995). They are able to bind lipids inducing increased levels of fecal steroid excretion (Wang et al. 1995). The interaction with soy protein replacements and digestibility of lipids cannot be overlooked, however in our study, the results showed no decrease in performance with either soy or soy protein concentrates, and the whole body proximate lipid composition was not significantly different among treatments.

Although no substantial negatives on growth performance were observed in the short duration of the current study, further investigations relating to the gut health of these fish, are necessary to rule out the possibility that longer term feeding is not detrimental to fish health. Multiple researchers have demonstrated that salmonids fed soybean meal for extended periods develop chronic enteritis (Olli et al. 1995; Olli and Krogdahl 1994) thus identifying possible differences in intestinal health between treatments may provide an improved understanding the effects and tolerances of this species to soy protein products.

Conclusions

In conclusion, Snake River cutthroat trout performed better with higher soy protein products inclusion levels. This data indicates there is no apparent issue when soybean protein is included up to 30% when rearing Snake River cutthroat trout. Further research is necessary to validate these results, and the soy protein sources could be further evaluated to measure anti-nutritional factors; the gastrointestinal tract needs to be investigated histologically to see if pathological differences among diets were present. Additionally, future research with other strains of cutthroat trout may show a greater sensitivity to plant protein products requiring a more cognizant decision when culturing those strains.

Acknowledgements

The authors wish to thank Jason Frost, Mark Portman, Clete Deshaser, Carly Stone, and Nick Leonard for their assistance with diet manufacturing, Matt Toner, Jason Ilgen, for their assistance with fish culture and sampling and Thomas O'Neill, Aaron Nistler and Omolola Betiku for their assistance with laboratory analyses. We would also like to thank the Bozeman Fish Health Lab for their collaborative efforts with collecting and analyzing sections of intestine for histological examination. Funding for the study was provided, in part, by the Western Regional Aquaculture Center through grant number 2012-38500-19657 from the US department of Agriculture National Institute of Food and Agriculture. Mention of trade names or commercial products in this article is solely for

the purpose of providing specific information and does not imply recommendation or endorsement by the authors, the U.S. Department of Agriculture or the U.S. Fish and Wildlife Service.

Table 4.1 Composition of the experimental formulation fed to juvenile Snake River cutthroat.

Ingredient ¹	Soybean Meal						Soy Protein Concentrate								
	0%	5%	10%	15%	30%	0%	5%	10%	15%	30%	0%	5%	10%	15%	30%
Soybean meal	0.0	5.0	10.0	15.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Soy Protein Concentrate	0.0	0.0	0.0	0.0	0.0	0.0	5.0	10.0	15.0	30.0	0.0	0.0	0.0	0.0	0.0
Chicken 42 – ADF	20.0	18.6	17.3	15.9	11.7	20.0	18.0	15.9	13.8	7.7	20.0	18.0	15.9	13.8	7.7
Sardine – CSF	30.0	27.9	25.9	23.8	17.6	30.0	26.9	23.8	20.7	11.5	30.0	26.9	23.8	20.7	11.5
Wheat flour	21.0	18.3	15.5	12.6	4.1	21.0	19.3	17.6	15.8	10.5	21.0	19.3	17.6	15.8	10.5
Menhaden fish oil	7.0	7.2	7.5	7.7	8.4	7.0	7.3	7.7	8.0	9.0	7.0	7.3	7.7	8.0	9.0
Poultry fat	0.0	0.2	0.5	0.7	1.4	0.0	0.3	0.7	1.1	2.1	0.0	0.3	0.7	1.1	2.1
Corn Protein Concentrate	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Spirulina	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Wheat gluten meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Krill meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C 35	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix ARS ²	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TM ARS 640 ³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Astaxanthin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Grobiotic A	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Taurine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline Cl 50%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Monocalcium Phosphate	2.0	2.3	2.7	3.1	4.3	2.0	2.5	3.1	3.7	5.4	2.0	2.5	3.1	3.7	5.4
DL-Methionine	0.3	0.3	0.4	0.4	0.6	0.3	0.4	0.4	0.5	0.8	0.3	0.4	0.4	0.5	0.8
Lysine HCl	1.5	1.7	2.0	2.2	3.0	1.5	1.9	2.2	2.6	3.7	1.5	1.9	2.2	2.6	3.7
Threonine	0.3	0.4	0.4	0.6	0.9	0.3	0.4	0.6	0.8	1.3	0.3	0.4	0.6	0.8	1.3
Analyzed Composition⁴															
% Crude protein	49.6	49.5	50.2	49.6	50.6	50.1	50.3	50.9	50.6	50.9	50.1	50.3	50.9	50.6	50.9
% Lipid	13.1	14.3	15.0	15.0	15.7	13.6	14.2	14.8	15.1	15.1	13.6	14.2	14.8	15.1	15.1
% Moisture	4.4	4.5	3.7	4.3	4.3	3.7	3.6	3.0	3.2	3.5	3.7	3.6	3.0	3.2	3.5
Gross energy kcal/g	5223	5221	5285	5237	5225	5235	5256	5290	5303	5218	5235	5256	5290	5303	5218

¹ Ingredient sources: List ingredient and then source ex fishmeal (Skretting, Toole, UT); Gavilon LLC,(Omaha, NE, USA), MGP Ingredients, Inc. (Atchison, KS, USA).

² Contributed per kg of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D₃, 2160 IU; vitamin E (as DL- α -tocopheryl-acetate), 1590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamin mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; vitamin B₁₂, 90 ug.

³ Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3; selenium, 0.4

⁴ Means on duplicate analyses on an as-fed basis.

Table 4.2 Growth of Snake River cutthroat juveniles^a fed either soy protein concentrate or soy bean meal at 0, 5, 10, 15, or 30% for 10 weeks.

Soy Type	Soy Level	Final fish weight (g)	Average Tank Gain (g)	Growth increase (%)	%BW/Da y	FCR	Survival
Soybean Meal	0	97.8 ^d	1396 ^d	250 ^c	2.9	1.8 ^a	100
	5	114.3 ^c	1629 ^c	301 ^c	2.8	1.6 ^a	97
	10	115.1 ^{bc}	1744 ^b	313 ^{bc}	2.4	1.4 ^c	100
	15	117.9 ^b	1760 ^b	324 ^b	2.9	1.6 ^{ab}	98
	30	131.3 ^a	1973 ^a	368 ^a	2.7	1.5 ^{bc}	97
Soy Protein Concentrate	0	97.8 ^d	1402 ^d	253 ^c	2.7	1.7 ^a	100
	5	105.1 ^c	1537 ^c	272 ^c	2.8	1.6 ^a	100
	10	118.8 ^{bc}	1810 ^b	320 ^{bc}	2.6	1.4 ^c	100
	15	123.1 ^b	1847 ^b	330 ^b	2.7	1.5 ^{ab}	98
	30	136.2 ^a	2099 ^a	371 ^a	2.6	1.4 ^{bc}	98
Pooled SEM		5.1	84	16	0.12	0.08	1.8
Pr>F		0.0003	<0.0001	0.0002	0.2379	0.0342	0.7695
Soy Type		0.7790	0.4810	0.8490	0.4712	0.5368	0.3765
Soy Level		<0.0001	<0.0001	<0.0001	0.0825	0.0044	0.5286
Type*Level		0.6019	0.7308	0.7801	0.6131	0.7315	0.8316

^aMeans of three tanks per diet.

^bProbability associated with the F-statistic.

^cA significant (P<0.05) main effect without significant interaction allowed data to be pooled; thus data within a column followed by different letters are significantly different when pooled as determined by Tukey's LSD.

Table 4.3 Body condition indices of Snake River cutthroat juveniles^a fed either soy protein concentrate or soy bean meal at 0,5,10,15, or 30% for 10 weeks.

Soy Type	Soy Level	VSI	HSI	MR
Soybean Meal	0	10.4	1.2	56.0bc
	5	9.9	1.1	56.3abc
	10	10.2	1.1	58.0a
	15	9.0	0.9	58.0ab
	30	9.6	0.9	54.3c
Soy Protein Concentrate	0	10.7	1.3	55.4bc
	5	9.5	1.1	56.3abc
	10	10.0	1.2	58.8a
	15	10.4	1.2	57.1ab
	30	10.8	1.1	55.6c
Pooled SEM		0.51	0.08	1.03
Pr>F ^b		0.3542	0.2420	0.1311
Soy Type		0.1795	0.0692	0.8903
Soy Level		0.4726	0.2558	0.0222
Type*Level		0.3141	0.5143	0.7963

^aMeans of five fish pools from each of two tanks per diet for the large tanks and five tanks per diet for the small tanks.

^bProbability associated with the F-statistic.

^cA significant (P<0.05) main effect of soy level without significant interaction allowed data to be pooled; thus data within columns followed by different letters are significantly different when pooled as determined by Tukey's LSD.

Table 4.4 Proximate composition of Snake River cutthroat juveniles^a fed either soy protein concentrate or soy bean meal at 0, 5, 10, 15, or 30% for 10 weeks.

Soy Type	Soy Level	% Protein	% Lipid	% Moisture	Energy (cal/g)
Soybean Meal	0	16.2	9.4	72.2	1789
	5	15.9	9.1	72.9	1784
	10	13.6	8.6	72.9	1747
	15	19.6	9	72.6	1779
	30	16.1	8.3	73.8	1686
Soy Protein Concentrate	0	15.8	9.2	72.9	1777
	5	16.0	8.3	72.3	1815
	10	13.9	9.2	73.4	1673
	15	16.8	8.8	72.5	1782
	30	16.4	9.9	72.4	1733
Pooled SEM		1.59	.60	.90	65.61
Pr>F ^b		0.4284	0.7402	0.9675	0.8531
Soy Type		0.6365	0.6131	0.777	0.9785
Soy Level		0.1371	0.3786	0.9258	0.4929
Type*Level		0.8337	0.8803	0.7863	0.9020

^aMeans of five fish pools from each of two tanks per diet for the large tanks and five tanks per diet for the small tanks.

^bProbability associated with the F-statistic.

^cA significant (P<0.05) main effect of soy level without significant interaction allowed data to be pooled; thus data within columns followed by different letters are significantly different when pooled as determined by Tukey's LSD.

References

- Aksnes, A., Mundheim, H., Toppe, J., & Albrektsen, S. (2008). The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets. *Aquaculture*, 275(1), 242-249.
- AOAC. 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.
- Arnesen, P., Brattas, L. E., Olli, J., & Krogdahl, A. (1989). Soybean carbohydrates appear to restrict the utilization of nutrients by Atlantic salmon (*Salmo salar* L.). In *The Current Status of Fish Nutrition in Aquaculture. The Proceedings of the Third International Symposium on Nutrition and Feeding in Fish. Tokyo University of Fisheries, Toba, Japan* (pp. 273-280).
- Baeverfjord, G., & Krogdahl, Å. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *Journal of Fish Diseases*, 19(5), 375-387.
- Bakke-McKellep, A. M., Press, C. M., Baeverfjord, G., Krogdahl, Å., & Landsverk, T. (2000). Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. *Journal of Fish Diseases*, 23(2), 115-127
- Barrows, F. T., Gaylord, T. G., Stone, D. A. J., & Smith, C. E. (2007). Effect of protein source and nutrient density on growth efficiency, histology and plasma amino acid concentration of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture research*, 38(16), 1747-1758.
- Bureau, D. P., Harris, A. M., & Young Cho, C. (1998). The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 161(1), 27-43.
- Buttle, L. G., Burrells, A. C., Good, J. E., Williams, P. D., Southgate, P. J., & Burrells, C. (2001). The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, (*Salmo salar*) and Rainbow trout, (*Oncorhynchus mykiss*), fed high levels of soybean meal. *Veterinary immunology and immunopathology*, 80(3), 237-244.
- Cheng, Z. J., & Hardy, R. W. (2003). Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 9(2), 77-83.

- Dale, O. B., Tørud, B., Kvellestad, A., Koppang, H. S., & Koppang, E. O. (2009). From chronic feed-induced intestinal inflammation to adenocarcinoma with metastases in salmonid fish. *Cancer research*, 69(10), 4355-4362.
- Davies, S. J., & Morris, P. C. (1997). Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. *Aquaculture Research*, 28(1), 65-74.
- De Francesco, M., Parisi, G., Médale, F., Lupi, P., Kaushik, S. J., & Poli, B. M. (2004). Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 236(1), 413-429.
- Ellis, A. E. (1995). Recent development in oral vaccine delivery systems. *Fish Pathology*, 30(4), 293-300.
- Eldridge, A. C., & Kwolek, W. F. (1983). Soybean isoflavones: effect of environment and variety on composition. *Journal of Agricultural and Food Chemistry*, 31(2), 394-396.
- Forster, I. (2002). Use of soybean meal in the diets of non-salmonid marine fish. *United Soybean Board. American Soybean Association. Available in <http://www.soyaqua.org>.*
- Fowler, L. G. (1980). Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. *The Progressive Fish-Culturist*, 42(2), 87-91.
- Franke, A. A., Custer, L. J., Cerna, C. M., & Narala, K. K. (1994). Quantitation of phytoestrogens in legumes by HPLC. *Journal of Agricultural and Food Chemistry*, 42(9), 1905-1913.
- Gatlin, D.M., F.T. Barrows, D. Bellis, P. Brown, J. Campen, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. RUSt, W. Sealey, D. Skonberg, E. Souza, D. Stone, R. Wilson, and E. Wurtele. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*, 38(6), 551-579.
- Gaylord, T. G., & Barrows, F. T. (2009). Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. *Aquaculture*, 287(1), 180-184.

- Gaylord, T. G., & Rawles, S. D. (2005). The Modification of Poultry By-product Meal for Use in Hybrid Striped Bass *Morone chrysops* × *M. saxatilis* Diets. *Journal of the World Aquaculture Society*, 36(3), 363-374.
- Gomes, E. F., Rema, P., Gouveia, A., & Teles, A. O. (1995). Replacement of fish meal by plant proteins in diets for rainbow trout (*Oncorhynchus mykiss*): effect of the quality of the fishmeal based control diets on digestibility and nutrient balances. *Water Science and Technology*, 31(10), 205-211.
- Hajen, W. E., Higgs, D. A., Beames, R. M., & Dosanjh, B. S. (1993). Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility. *Aquaculture*, 112(4), 333-348.
- Hardy, R.W. 2002. Webster, C. D., & Lim, C. (Eds.). (2002). *Nutrient requirements and feeding of finfish for aquaculture*. Cabi.
- Kaushik, S. J., Cravedi, J. P., Lalles, J. P., Sumpter, J., Fauconneau, B., & Laroche, M. (1995). Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 133(3), 257-274.
- Kindschi, G. A., Myrick, C. A., Barrows, F. T., Toner, M., Fraser, W. C., Ilgen, J., & Beck, L. (2009). Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture*, 71(4), 325-329.
- Krogdahl, Å., Lea, T. B., & Olli, J. J. (1994). Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A: Physiology*, 107(1), 215-219.
- Lansard, M., Panserat, S., Plagnes-Juan, E., Dias, K., Seiliez, I., & Skiba-Cassy, S. (2011). L-leucine, L-methionine, and L-lysine are involved in the regulation of intermediary metabolism-related gene expression in rainbow trout hepatocytes. *The Journal of nutrition*, 141(1), 75-80.
- Liener, I. E. (1994). Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science & Nutrition*, 34(1), 31-67.
- Lusas, E. W., & Riaz, M. N. (1995). Soy protein products: processing and use. *The Journal of nutrition*, 125(3 Suppl), 573S-580S.

- Mambrini, M., Roem, A. J., Carvedi, J. P., Lalles, J. P., & Kaushik, S. J. (1999). Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss*. *Journal of animal science*, 77(11), 2990-2999.
- Médale, F., Boujard, T., Vallée, F., Blanc, D., Mambrini, M., Roem, A., & Kaushik, S. J. (1998). Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. *Aquatic Living Resources*, 11(04), 239-246.
- Myrick, C. A. (2008). Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout. *Western Regional Aquaculture Center Annual Progress Report, Colorado State University, Fort Collins Colorado*.
- National Research Council (NRC). 2011. Nutrient Requirements of Fish. National Academy Press, Washington (2011).
- Nordrum, S., Bakke-McKellep, A. M., Krogdahl, Å., & Buddington, R. K. (2000). Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar L.*) and rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 125(3), 317-335.
- Olli, J., Krogdahl, A., & Berg-Lea, T. (1989). Effects of soybean trypsin inhibitor activity on nutrient digestibility in salmonids fed practical diets containing various soybean meals. In *Proc. Third Int. Symp. on Feeding and Nutrition in Fish* (Vol. 28, pp. 263-271).
- Olli, J. J., & Krogdahl, Å. (1995). Alcohol soluble components of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar L.* *Aquaculture Research*, 26(11), 831-835.
- Olli, J. J., Krogdahl, Å., van den Ingh, T. S., & Brattås, L. E. (1994). Nutritive value of four soybean products in diets for Atlantic salmon (*Salmo salar, L.*). *Acta Agriculturae Scandinavica, Section A-Animal Science*, 44(1), 50-60.
- Ott, L. (1977). An introduction to statistical methods and data analysis. Duxbery Press, North Scituate, Massachusetts, USA.
- Pongmaneerat, J., & Watanabe, T. (1993). Nutritional evaluation of soybean meal for rainbow trout and carp. *Bulletin of the Japanese Society of Scientific Fisheries (Japan)*.

- Refstie, S., Storebakken, T., & Roem, A. J. (1998). Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fishmeal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture*, 162(3), 301-312.
- Refstie, S., Korsøen, Ø. J., Storebakken, T., Baeverfjord, G., Lein, I., & Roem, A. J. (2000). Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 190(1), 49-63.
- Rodehutsord, M., Borchert, F., Gregus, Z., Pack, M., & Pfeffer, E. (2000). Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*): 1. Effect of dietary crude protein level. *Aquaculture*, 187(1), 163-176.
- Rumsey, G. L., Endres, J. G., Bowser, P. R., Earnest-Koons, K. A., Anderson, D. P., & Siwicki, A. K. (1995). Soy protein in diets of rainbow trout: effects on growth, protein absorption, gastrointestinal histology, and nonspecific serologic and immune response. *Nutrition and utilization technology in aquaculture*, 166-188.
- Rumsey, G. L., Hughes, S. G., & Winfree, R. A. (1993). Chemical and nutritional evaluation of soya protein preparations as primary nitrogen sources for rainbow trout (*Oncorhynchus mykiss*). *Animal feed science and technology*, 40(2), 135-151.
- Sealey, W. M., Barrows, F. T., Smith, C. E., Overturf, K., & LaPatra, S. E. (2009). Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout (*Oncorhynchus mykiss*) to utilize high levels of soybean meal during grow-out. *Aquaculture*, 293(3), 195-203.
- Snyder, G. S., Gaylord, T. G., Barrows, F. T., Overturf, K., Cain, K. D., Hill, R. A., & Hardy, R. W. (2012). Effects of carnosine supplementation to an all-plant protein diet for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 338, 72-81.
- Tsukamoto, C., Shimada, S., Igita, K., Kudou, S., Kokubun, M., Okubo, K., & Kitamura, K. (1995). Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *Journal of Agricultural and Food Chemistry*, 43(5), 1184-1192.
- Tukey, J. (1953). *The problem of multiple comparisons*, Princeton University, Princeton, New Jersey, USA.

- Van den Ingh, T. S. G. A. M., Krogdahl, Å., Olli, J. J., Hendriks, H. G. C. J. M., & Koninkx, J. G. J. F. (1991). Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture*, 94(4), 297-305.
- Wang, M. F., Yamamoto, S., Chung, H. M., Chung, S. Y., Miyatani, S., Mori, M., ... & Sugano, M. (1995). Antihypercholesterolemic effect of undigested fraction of soybean protein in young female volunteers. *Journal of nutritional science and vitaminology*, 41(2), 187-195.
- D. Webster, C., Tidwell, J. H., Goodgame, L. S., Yancey, D. H., & Mackey, L. (1992). Use of soybean meal and distillers grains with solubles as partial or total replacement of fishmeal in diets for channel catfish, *Ictalurus punctatus*. *Aquaculture*, 106(3), 301-309.
- Yamamoto, T., Sugita, T., & Furuita, H. (2005). Essential amino acid supplementation to fish meal-based diets with low protein to energy ratios improves the protein utilization in juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 246(1), 379-391.

CHAPTER FIVE

CONCLUSIONS

Identification of a specific pellet type preference for Snake River cutthroat trout could allow producers to select a diet for use that would allow for better growth characteristics, and more uniform growth results. The use of plant proteins that can mitigate feed price fluctuations and reduce the use of fishmeal for commercial trout production, soy based protein sources have been widely studied and do proved adequate when essential limiting amino acids are supplemented, however the affects need to be looked at closer to see if detrimental side effects exist when rearing not target salmonids with commercial production diets. To achieve these goals, the first objective of this thesis research was to investigate factors that affect pellet type preference when rearing Westslope and Snake River cutthroat trout. The second objective evaluated the use of soy protein sources at varying levels to see if the response was similar to closely related salmonids.

Previous research has shown that pellet type alters feeding behavior and growth of salmonids but were limited by the use of commercial closed formula diets with differing compositions. Results from Experiment 1 further those results by demonstrating that when keeping a consistent formulation, a flake feed is not adequate for rearing cutthroat trout at this life stage. The current study demonstrated a beneficial effect of the semi moist diet on growth performance of cutthroat trout, this diet was significantly superior in regards to growth performance when fed to Snake Rive cutthroat trout.

As commercial rainbow trout diets transition to higher soy inclusion levels, the effects on non-target species that utilize these diets maybe negatively affected. Results from Experiment 2 demonstrated that the inclusion of soy protein products up to 30% did not affect growth for this 12 week period. However, this needs to be looked at again to ensure there were no other variables affecting the growth performance. Histological data is being interpreted by the Bozeman Fish Health Lab staff to evaluate soy inclusion level effects on intestinal health. These results will aid in the development of a species-specific diet, and help producers chose commercially available diets for rearing this species throughout the life cycle of the species.

REFERENCES CITED

- Alanärä, A. (1996). The use of self-feeders in rainbow trout (*Oncorhynchus mykiss*) production. *Aquaculture*, 145(1), 1-20.
- Alanärä, A. (1992). The effect of time-restricted demand feeding on feeding activity growth and feed conversion in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 108(3), 357-368.
- Aloisi, D. B. (1994). Growth of hatchery-reared lake trout fed by demand feeders. *The Progressive Fish-Culturist*, 56(1), 40-43.
- Arndt, R. E., Wagner, E. J., Routledge, M. D., & Bradwisch, Q. A. (1998). Hatchery performance and fish health of cutthroat and rainbow trout hand-fed or demand-fed a floating feed. *Journal of applied aquaculture*, 8(3), 41-51.
- Arndt, R. E., & Wagner, E. J. (2007). Enriched Artemia and probiotic diets improve survival of Colorado River cutthroat trout larvae and fry. *North American journal of aquaculture*, 69(2), 190-196.
- Azevedo, P. A., Leeson, S., Cho, C. Y., & Bureau, D. P. (2004). Growth and feed utilization of large size rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) reared in freshwater: diet and species effects, and responses over time. *Aquaculture nutrition*, 10(6), 401-411.
- Bear, E. A., McMahon, T. E., & Zale, A. V. (2007). Comparative thermal requirements of westslope cutthroat trout and rainbow trout: implications for species interactions and development of thermal protection standards. *Transactions of the American Fisheries Society*, 136(4), 1113-1121.
- Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6, Bethesda, Maryland.
- Dwyer, W. P., & Piper, R. G. (1987). Atlantic salmon growth efficiency as affected by temperature. *The Progressive Fish-Culturist*, 49(1), 57-59.
- Dwyer, W. P., Piper, R. G., & Smith, C. E. (1983). Brook trout growth efficiency as affected by temperature. *The Progressive Fish-Culturist*, 45(3), 161-163.
- Edsall, D. A. (1986). Comparison of semi-moist and dry starter diets for cutthroat trout.
- Edsall, D. A. (1987). Evaluation of starter diets for Snake River cutthroat trout. *The Progressive Fish-Culturist*, 49(2), 93-95.

- Fraley, J. (2002). Can hatcheries help conserve the westslope cutthroat. *Montana Outdoors*, 33(2), 14-19.
- Hardy, R. W. (2001). Fish Feeds and Nutrition One Size Doesn't Fit All. *Aquaculture Magazine-Arkansas*, 27(5), 78-81.
- Hilderbrand, R. H. (2002). Simulating supplementation strategies for restoring and maintaining stream resident cutthroat trout populations. *North American Journal of Fisheries Management*, 22(3), 879-887.
- Hilton, J. W., Cho, C. Y., & Slinger, S. J. (1977). Factors affecting the stability of supplemental ascorbic acid in practical trout diets. *Journal of the Fisheries Board of Canada*, 34(5), 683-687.
- Hilton, J. W., Cho, C. Y., & Slinger, S. J. (1981). Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.). *Aquaculture*, 25(2), 185-194.
- Johnstone, H. C., & Rahel, F. J. (2003). Assessing temperature tolerance of Bonneville cutthroat trout based on constant and cycling thermal regimes. *Transactions of the American Fisheries Society*, 132(1), 92-99.
- Kindschi, G. A. (1984). Notes on two feed types and methods for steelhead trout production. *The Progressive Fish-Culturist*, 46(1), 44-47.
- Kindschi, G. A., Myrick, C. A., Barrows, F. T., Toner, M., Fraser, W. C., Ilgen, J., & Beck, L. (2009). Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture*, 71(4), 325-329.
- Kindschi, G. A., & Koby Jr, R. F. (1994). Performance and oxygen consumption of Snake River cutthroat trout reared at four densities with supplemental oxygen. *The Progressive Fish-Culturist*, 56(1), 13-18.
- Luecke, C. (1986). Ontogenetic changes in feeding habits of juvenile cutthroat trout. *Transactions of the American Fisheries Society*, 115(5), 703-710.
- Liknes, G. A. (1988). Westslope Cutthroat Trout in Montana: Life History, Status, and Management.
- Lovell, R. T., & Lim, C. (1978). Vitamin C in pond diets for channel catfish. *Transactions of the American Fisheries Society*, 107(2), 321-325.

- Maskill, Brian. *Cutthroat Trout Interview* 15 march 2012. march . Personal interview.
- McCullough, D. A. (1999). A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook salmon.
- Myrick, C. A. (2008). Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout. *Western Regional Aquaculture Center Annual Progress Report, Colorado State University, Fort Collins Colorado*.
- Nowak, G. M., Tabor, R. A., Warner, E. J., Fresh, K. L., & Quinn, T. P. (2004). Ontogenetic shifts in habitat and diet of cutthroat trout in Lake Washington, Washington. *North American Journal of Fisheries Management*, 24(2), 624-635.
- NRC (National Research Council). 2011. Nutrient requirements of fish. National Academies Press, Washington, D.C.
- Piper, R. G. (1986). *Fish hatchery management*. US Dept. of the Interior, Fish and Wildlife Service.
- Rubidge, E. M., & Taylor, E. B. (2005). An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. *Conservation Genetics*, 6(3), 369-384.
- Shepard, B. B., May, B. E., & Urie, W. (2005). Status and conservation of westslope cutthroat trout within the western United States. *North American Journal of Fisheries Management*, 25(4), 1426-1440.
- Shepard, B. B., Sanborn, B., Ulmer, L., & Lee, D. C. (1997). Status and risk of extinction for westslope cutthroat trout in the upper Missouri River basin, Montana. *North American Journal of Fisheries Management*, 17(4), 1158-1172.
- Smith, C. E., Dwyer, W. P., & Piper, R. G. (1983). Effect of water temperature on egg quality of cutthroat trout. *The Progressive Fish-Culturist*, 45(3), 176-178.
- Smith, M. A., Hubert, W. A., & Barrows, F. T. (2004). Failure of a plant-and-krill-based diet to affect the performance of Yellowstone Cutthroat trout broodfish. *North American Journal of Aquaculture*, 66(1), 61-69.
- Springer, C. 2004. Native trout conservation in the U.S. West. *Hatchery International* 5(6):16-18.

- Statler, D. P. (1982). Use of self-feeders for rearing steelhead trout at Dworshak National Fish Hatchery. *The Progressive Fish-Culturist*, 44(4), 195-195.
- Stradmeyer, L. (1989). A behavioural method to test feeding responses of fish to pelleted diets. *Aquaculture*, 79(1), 303-310.
- Stradmeyer, L. (1989). A behavioural method to test feeding responses of fish to pelleted diets. *Aquaculture*, 79(1), 303-310.
- McMahon, Tom. "Ichthyology." Montana State University. Montana, Bozeman. 16 February, 2012. Lecture.
- Thurrow, R. F., Lee, D. C., & Rieman, B. E. (1997). Distribution and status of seven native salmonids in the interior Columbia River basin and portions of the Klamath River and Great basins. *North American Journal of Fisheries Management*, 17(4), 1094-1110.
- Tipping, J. M., Rathvon, R. L., & Moore, S. T. (1986). Use of demand feeders on large steelhead rearing ponds. *The Progressive Fish-Culturist*, 48(4), 303-304.
- Wagner, E. J., Arndt, R. E., & Roubidoux, R. (2006). The effect of temperature changes and transport on cutthroat trout eggs soon after fertilization. *North American journal of aquaculture*, 68(3), 235-239.
- Wagner, E. J., Routledge, M. D., & Intelmann, S. S. (1996). Assessment of demand feeder spacing on hatchery performance, fin condition, and size variation of rainbow trout *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*, 27(1), 130-136.
- Wagner, E. J., Jeppsen, T., Arndt, R., Routledge, M. D., & Bradwisch, Q. (1997). Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin erosion, and general health and condition. *The Progressive Fish-Culturist*, 59(3), 173-187.