THESIS

EVALUATING DIETARY AND BEHAVIORAL IMPACTS OF COMMERCIAL-TYPE DIETS ON THE GROWTH AND ANTI-PREDATOR RESPONSES OF SNAKE RIVER CUTTHROAT TROUT (*Oncorhynchus clarkii behnkei*)

Submitted by

Cheyenne Elizabeth Owens

Department of Fish, Wildlife, and Conservation Biology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2016

Master's Committee:

Advisor: Christopher A. Myrick

Nicole K.M. Vieira
Lisa M. Angeloni

ABSTRACT

EVALUATING DIETARY AND BEHAVIORAL IMPACTS OF COMMERCIAL-TYPE DIETS ON THE GROWTH AND ANTI-PREDATOR RESPONSES OF SNAKE RIVER CUTTHROAT TROUT (*Oncorhynchus clarkii behnkei*)

Cutthroat trout (*Oncorhynchus clarkii*) are raised for restoration stocking and to provide boutique sport fishing opportunities. Because of limited cutthroat-specific culture information, cutthroat trout have been raised using diets and techniques developed for rainbow trout (*Oncorhynchus mykiss*), resulting in inconsistent growth performance. There is also evidence that intensive culturing may diminish anti-predator behavior in salmonids, which has not been tested in cutthroat trout. A brief overview of the state of cutthroat trout is described in chapter one of this thesis.

The second chapter of this thesis describes a 6-month feeding trial conducted on juvenile Snake River cutthroat trout (*Oncorhynchus clarkii behnkei*) fed six different feed formulations. Two floating control diets were chosen for this study (Skretting Classic Trout and Skretting Steelhead), along with three floating commercial-type formulations with varying crude protein (CP) and crude lipid (CL) levels (40 CP:12CL, 45CP:16CL, and 45CP:24CL) and one floating experimental formulation (40CP:16CL diet with lysine, methionine and threonine balanced to match the 45CP:16CL diet – BFTC Experimental). Diet significantly ($P<0.05$) affected final average fish weight, with fish fed Skretting Steelhead, BFTC Experimental, and 45CP:24CL weighing significantly more than fish fed 40CP:12CL. Proximate composition was also altered

by diet, with fish fed 45CP:24CL having significantly higher crude energy levels than fish fed 40CP:12CL and Skretting Classic Trout. In a simple cost analysis, it was found that the BFTC Experimental diet provided the lowest cost per pound of fish out of all diets. The results indicate that diets with greater than 40% protein and 12% lipid provide the greatest growth in juvenile Snake River cutthroat trout, and that amino acid balanced diets provide a cost efficient option for cutthroat trout growers.

The third chapter of this thesis describes a study wherein fish from the diet study were divided into one of two different size classes (small [12 ± 2.5 cm TL], and large [20 ± 2.5 cm TL]) and observed during open field testing and during exposure to a novel avian predator model (great blue heron, *Ardea herodias*). Additional testing was run separately on a medium size class [16 ± 2.5 cm TL]. Small fish were significantly ($P<0.05$) less likely to freeze during open field tests than large fish and potentially more likely to dart ($P=0.0652$) than medium fish during simulated predator attacks. Significant differences in freezing response between small and large fish fed different diets were observed ($P<0.05$), with fish fed 45CP:16CL and BFTC Experimental showing a higher probability of freezing than fish fed Skretting Steelhead. Potential differences in darting response between medium fish fed different diets were also observed ($P=0.0825$), suggesting that differences in ingredients or ingredient inclusion levels between experimental and control diets had subtle effects on behavior. The results indicate that hatchery-reared cutthroat trout do exhibit anti-predator behaviors in response to a novel predator, however further research is necessary to determine if these behaviors differ from those exhibited by wild cutthroat trout.

ACKNOWLEDGEMENTS

This project was supported by Western Regional Aquaculture Center Grant nos. #2012-38500-19657, and #2014-38500-22309 from the USDA National Institute of Food and Agriculture. Additional funding was provided by the Kalamazoo Community Foundation George L. Disborough Trout Unlimited Research Grant, Robert J. Behnke Rocky Mountain Flycasters Environmental Fellowship, and the U.S. Fish and Wildlife Service Directorate Resource Assistant Fellows Program. All fish were handled and treated in accordance with the Colorado State University Institutional Animal Care and Use Committee (Protocols #14-4889A and 15-5822A) and USFWS procedures according to the Guidelines for Use of Fishes in Research (2004).

I would like to thank the U.S. Fish and Wildlife Service Bozeman Fish Technology Center for providing supplies, lab space, and manufacturing experimental feeds used for this research. In particular, I thank Dr. Bob Muth, Matt Toner, Cal Fraser, Jason Ilgen, Dr. Gibson Gaylord, Anthony Rodriguez-Vargas, and Leif Halverson for their support throughout this research. I especially thank Zachariah Conley for caring for the Snake River cutthroat trout (*Oncorhychus clarkii behnkei*) used in this research on my behalf and for his guidance in proximate composition laboratory procedures; and Aly Hink for her many dedicated hours of behavioral analysis. In addition, I thank Jackie Zimmerman for her feed expertise, Dr. Ann Hess for statistical guidance; and Alyssa Graziano, Tyler Swarr, Jordan Anderson, Brian Avila, Chris Kopack, Stark Cubs, Chris Kotalik, Adam Herdrich, Estevan Vigil, Kyle Christianson, and Hannah Riedl for their advice and support.

I thank my major advisor, Dr. Chris Myrick, and Dr. Wendy Sealey for their mentorship and guidance. As a minority in fisheries, I cannot express the impact that both of my mentors have made on my career outlook; none of this research would be possible without them and I am eternally grateful for the opportunities they have provided me. I also thank my committee members, Dr. Lisa Angeloni and Dr. Nicole Vieira, for lending their expertise to this research and for their advocacy.

TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGEMENTS iv
CHAPTER 1: THE STATE OF CUTTHROAT TROUT 1
Tables and Figures 4
REFERENCES 5
CHAPTER 2: GROWTH OF JUVENILE SNAKE RIVER CUTTHROAT TROUT FED COMMERCIAL-TYPE DIETS 8
1. Summary 8
2. Introduction 8
3. Methods 11
3.1 Experimental animals 11
3.2 Diet formulation and production 11
3.3 Experimental design 12
3.4 Fish sampling 13
3.5 Proximate composition analyses 14
3.6 Statistical analyses 14
4. Results 15
4.1 Growth parameters, nutrient utilization and survival rates 15
4.2 Proximate composition and condition indices 15
4.3 Cost analysis 16
5. Discussion 16
6. Conclusions 19
Tables and Figures 20
REFERENCES 26
CHAPTER 3: EVALUATION OF SUBADULT SNAKE RIVER CUTTHROAT TROUT: DOES DIET OR SIZE INFLUENCE BEHAVIOR? 32
1. Summary 32
2. Introduction 32
3. Methods 35
3.1 Experimental animals 35
3.2 Experimental design 36
3.3 Behavioral analyses 38
3.4 Statistical analyses 38
4. Results 39
4.1 Open field tests 39
4.2 Predator exposures 40
5. Discussion 41
6. Conclusions 45
Tables and Figures 46
REFERENCES 55

CHAPTER 1: THE STATE OF CUTTHROAT TROUT

Cutthroat trout (*Oncorhynchus clarkii*) are native to the western United States and have the greatest historic North American distribution of the native Pacific trout species (Behnke, 2002). Currently, there are fourteen recognized subspecies (Figure 1.1). Most cutthroat trout subspecies have experienced severe population declines, with four subspecies previously petitioned for listing under the Endangered Species Act (ESA), five subspecies protected under the ESA, and two subspecies recognized as extinct (Behnke, 2002). Although habitat fragmentation and degradation have contributed to the declines in native cutthroat trout, the primary cause of population contractions are interactions with non-native salmonids (Behnke, 2002; Peterson et al., 2008). Non-native salmonids can hybridize, outcompete, and prey upon cutthroat trout. For example, rainbow trout (*Oncorhynchus mykiss*) readily hybridize and outcompete cutthroat trout (Young, 1995; Behnke, 1992). Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) show lower growth rates when sympatric with cutthroat-rainbow hybrids (Seiler and Kelley, 2009). Both rainbow trout and hybrids have higher sustained swimming ability (Seiler and Keeley, 2007*a*) and superior foraging ability (Seiler and Keeley, 2007*b*) than Yellowstone cutthroat trout, which contribute to their competitive advantage. Lake trout (*Salvelinus namaycush*) prey upon Yellowstone cutthroat trout, posing a serious threat to the existence of these indigenous trout in Yellowstone Lake (Ruzycki et al., 2003). The introduction of brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) into the western United States led to the frequent displacement of cutthroat trout due to competition and predation (Peterson et al., 2004; Behnke, 2002).

In order to reverse cutthroat trout population declines, public natural resource management agencies have developed recovery plans that frequently include restoring or supplementing wild populations with hatchery-reared cutthroat trout. This has been done with nine of the cutthroat trout subspecies (Figure 1.1) (U.S. Fish and Wildlife Service 1995, 1998, 2001; Alves et al., 2004; Brandt, 2009; Costello, 2008; Kindschi et al., 2009; Ham et al., 2015*a*).

Success of conservation efforts that include population supplementations are predicated on the ability of aquaculturists to develop hatchery strains of cutthroat which can survive wild introduction. However, cutthroat trout culture is still limited in scope, relative to the much more widespread and large-scale production of rainbow trout, and the development of cutthroat-specific procedures has lagged behind that of more commonly produced species. This has forced hatchery personnel to adopt rearing conditions and techniques optimized for rainbow trout, with inconsistent and suboptimal growth and survival rates of hatchery-reared cutthroat trout (e.g., Bosakowski and Wagner, 1994a, 1994b; Kindschi et al., 2009; Brandt, 2009; Myrick and Fornshell, 2011). Improving the hatchery performance of cutthroat trout could increase the survival of this species post-stocking, an important component of restoration efforts (Brandt, 2009).

A major factor influencing the survival of stocked salmonids is predation (Biro et al., 2004), so it is important that stocked cutthroat trout retain the ability to recognize and avoid predators. However, recent behavioral studies have shown that captive-reared rainbow trout exhibit higher-risk foraging behavior than their wild counterparts (Biro et al., 2004) and may exhibit reduced anti-predator behavior (Brown and Smith, 1997). Thus, it is possible that standard rainbow trout rearing techniques may not prepare cutthroat trout for long-term survival under field conditions. Knowing whether hatchery-reared cutthroat trout display anti-predator behavior is vital to the success of restoration efforts for cutthroat trout, in which long-term survival of stocked individuals is paramount.

Tables and Figures

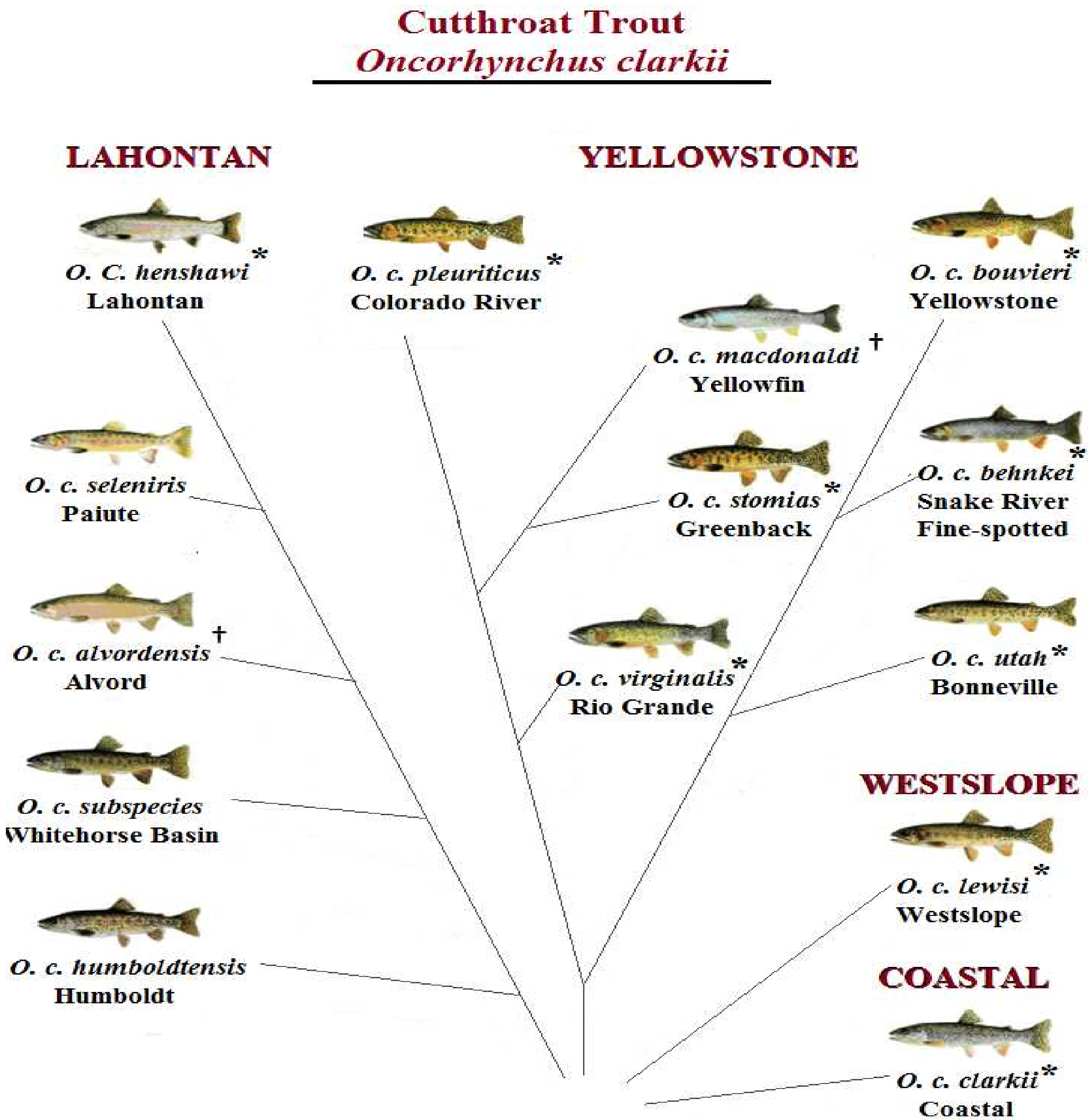

Figure 1.1. Phylogenetic map of cutthroat trout (*Oncorhynchus clarkii*) adapted from Behnke (2002). Asterisks indicate subspecies that have been cultured in public hatcheries (U.S. Fish and Wildlife Service 1995, 1998, 2001; Alves et al., 2004; Brandt, 2008; Costello, 2008; Kindschi et al., 2009; Ham et al., 2015*a*). Cutthroat trout images used with permission of Joseph R. Tomelleri.

REFERENCES

Alves, J., Krieger, D., Nesler, T., 2004. Conservation plan for Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) in Colorado. Colorado Division of Wildlife, Aquatic Wildlife Section, Denver, Colorado, USA.

Behnke, R.J., 2002. Trout and salmon of North America. The Free Press, New York, USA.

Behnke R.J., 1992. Native trout of western North America. American Fisheries Society 429 monograph no. 6, USA.

Biro, P.A., Abrahams, M.V., Post, J.R., Parkinson, E.A., 2004. Predators select against high growth rates and risky behavior in domestic trout populations. Proceedings of the Royal Society of London Biology 271, 2233–2237.

Brandt, M.M., 2009. Optimal starter diets and culture conditions for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). M.S. Thesis. Colorado State University, Fort Collins, CO.

Brown, G.E., Smith, J.F., 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 75, 1916– 1922.

Bosakowski, T., Wagner, E.J., 1994a. A survey of trout fin erosion, water quality, and rearing conditions at state fish hatcheries in Utah. Journal of the World Aquaculture Society 25, 308– 316.

Bosakowski, T., Wagner, E.J., 1994b. Assessment of fin erosion by comparison of relative fin length in hatchery and wild trout in Utah. Canadian Journal of Fisheries and Aquatic Sciences 51, 636– 641.

Costello, A.B., 2008. The statues of coastal cutthroat trout in British Columbia. Coastal Cutthroat Trout Symposium: Status, Management, Biology, and Conservation. Oregon Chapter, American Fisheries Society, Portland, USA.

Ham, B.R., Barrows, F.T., Huttinger, A., Duff, G.C., Yeoman, C.J., Maskill, M.G., Sealey, W.M., 2015. Evaluation of dietary soy sensitivity in Snake River cutthroat trout. North American Journal of Aquaculture 77 (2), 195– 2015

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.

Myrick, C.A., Fornshell, G., 2014. Early rearing of cutthroat trout. Western Regional Aquaculture Center, Seattle, Washington, USA.

Peterson, D.P., Fausch, K.D., Watmough, J., Cunjak, R.A., 2008. When eradication is not an option: modeling strategies for electrofishing suppression of non– native nonnative brook trout to foster persistence of sympatric native cutthroat trout in small streams. North American Journal of Fisheries Management 28 (6), 1847– 1867.

Peterson, D.P., Fausch, K.D., White, G.C., 2004. Population ecology of an invasion: effects of nonnative brook trout on native cutthroat trout. Ecological Applications 14, 754–772.

Ruzycki, J.R., Beauchamp, D.A., Yule, D.L., 2003. Effects of introduced lake trout on native cutthroat trout in Yellowstone Lake. Ecological Applications 13, 23– 37.

Seiler, S.M., Keeley, E.R., 2009. Competition between native and introduced salmonid fishes: cutthroat trout have lower growth rate in the presence of cutthroat-rainbow trout hybrids. Canadian Journal of Fisheries and Aquatic Sciences 66, 133– 141.

Seiler, S.M., Keeley, E.R., 2007*a*. Morphological and swimming stamina differences between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), rainbow trout (*Oncorhynchus mykiss*) and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 64, 127– 135.

Seiler, S.M., Keeley, E.R., 2007*b*. A comparison of aggressive and foraging behavior between juvenile cutthroat trout, rainbow trout, and F1 hybrids. Animal Behaviour. 74, 1805–1812.

U.S. Fish and Wildlife Service, 2001. Status review for Bonneville cutthroat trout (*Oncorhynchus clarki utah)*. U.S. Fish and Wildlife Service, Regions 1 and 6, Denver, Colorado, and Portland, Oregon, USA.

U.S. Fish and Wildlife Service, 1998. Greenback cutthroat trout recovery plan. U.S. Fish and Wildlife Service, Region 6, Denver, Colorado, USA.

U.S. Fish and Wildlife Service, 1995. Recovery plan for Lahontan cutthroat trout. U.S. Fish and Wildlife Service, Region 1, Portland, Oregon, USA.

Young, M.K, (ed.), 1995. Conservation assessment for inland cutthroat trout. US Forest Service, Fort Collins, CO, USA. Gen. Tech. Rep. RM-GTR-256.

CHAPTER 2: GROWTH OF JUVENILE SNAKE RIVER CUTTHROAT TROUT FED COMMERCIAL-TYPE DIETS

1. Summary

A six–month feeding trial was conducted to evaluate the effects of feed formulation on growth performance and proximate composition of Snake River cutthroat trout (*Oncorhynchus clarkii behnkei*) (initial body weight: 8.6 ± 2.0 g). Two commercially available control diets and four experimental diets formulated with varying levels of crude protein (CP) and crude lipid (CL) were used (40CP:12CL, 45CP:16CL, 45CP:24CL, 40CP:16CL amino acid balanced to equal 45CP:16CL [BFTC Experimental]). The results showed that fish fed diets with more than 40% crude protein and 12 % crude lipid had the highest values of final body weight, final body length, specific growth rate, and weight gain and lowest feed conversion ratios. Energy contents were significantly higher in fish fed the 45CP:24CL diet than fish fed the 40CP:12CL or its corresponding control diet. Cost analysis suggests that the most economical diet in terms of cost per kilogram of fish was the BFTC Experimental diet, which corresponds to commercially available amino acid balanced diets. Based on these results, growers should consider using a 45CP:16CL diet or higher to improve fish growth, proximate composition, and production efficiency when culturing Snake River cutthroat trout.

2. Introduction

There has been an increased interest over the years in the commercial propagation of native fish species, in particular native salmonids. Cutthroat trout (*Oncorhynchus clarkii*) are native to western North America and have traditionally been reared in state and federal hatcheries to conserve, restore or supplement declining natural populations. Recently, however, a growing number of private facilities are also culturing cutthroat trout for recovery programs and for recreational fishing (Kindschi et al., 2009). Cutthroat trout are viewed as a novel or boutique species and thus can command a premium price compared to the more common rainbow trout (*Oncorhynchus mykiss*).

Snake River cutthroat trout (*Oncorhynchus clarkii behnkei*), a subspecies native to the Upper Snake River in Wyoming and Idaho, have been widely stocked throughout the western United States and are the most common commercially cultured subspecies (Behnke, 2002). Snake River cutthroat trout are popular sport fish that are favored by fisheries management agencies because their distinctive fine-spotted color pattern makes it easy to distinguish them from other cutthroat trout (Myrick and Fornshell, 2011). They are also commonly used as one of the parents of the popular "cuttbow", a hybrid between rainbow trout and cutthroat trout.

Although the earliest instance of cutthroat trout culture occurred only a few decades after the earliest instance of rainbow trout culture (Behnke, 1992; Webster and Lim, 2002), advances in cutthroat trout culture have been lacking. The world-wide propagation of rainbow trout has brought continued refinement of spawning and rearing techniques (Piper et al., 1982), genetic selection for faster growth (Hulata, 2001), and nutritional requirements leading to rainbow trout-specific feeds (NRC, 2011). In comparison, cutthroat trout culture is of limited scope, so an equivalent level of development and research attention does not yet exist.

Due to the lack of cutthroat-specific culture information, hatchery personnel draw upon rainbow trout rearing conditions and techniques. However, inconsistent and sub-optimal growth, survival, and condition have repeatedly been reported using this approach (e.g., Bosakowski and Wagner, 1994a, 1994b; Kindschi et al., 2009). A number of studies in recent years have focused on optimizing specific elements of cutthroat trout culture in order to improve our ability to culture them. These studies have looked at rearing density (Kindschi and Koby, 1994; Wagner et al., 1997; Brandt, 2009), thermal preference (Bear et al., 2007; Johnson and Rahel, 2003), and diet development (Kindschi et al., 2009; Brandt, 2009; Ham et al., 2015*a,b*). Most of the published diet studies focused on improving the growth and survival of cutthroat trout fry and juveniles, with mixed results. Arndt and Wagner (2007) improved the survival of first-feeding Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus)* using *Lactobacillus*-enriched feeds, while Brandt (2009) improved both growth and survival in the same strain by supplementing premium salmonid diets with *Artemia*. Although switching broodstock from a fishmeal-based to a plant- and krill-based diet did not improve the survival of embryos to swim-up (Smith et al., 2004), Ham et al. (2015*a*) found that diets containing dietary soybean meal or soy protein concentrate provided greater growth of juvenile Snake River cutthroat trout than non-inclusive diets, albeit with intestinal inflammation present at high inclusion levels.

Physical feed characteristics can also influence the growth of cutthroat trout, with flake feeds shown to be inadequate complete feeds for juvenile stage fish (Ham et al., 2015*b*). Without cutthroat-specific diets available, studies have worked with diets formulated for other salmonids. Kindschi et al. (2009) found that a premium salmonid diet provided the highest growth and survival of Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) fry and Snake River cutthroat trout fry. These findings were reproduced at a production facility with fry, however Myrick et al. (2010) found that once the juveniles were transitioned to a regular rainbow trout production diet, a formulation typical for rainbow trout grow-out, growth performance decreased. With limited investigation on the long-term growth of cutthroat trout under culture conditions, additional research is needed to determine appropriate diets for cutthroat trout during the grow-out production period.

The purpose of this study was to determine whether existing commercial trout or salmon production diets can reduce or prevent the growth reduction observed in Snake River cutthroat trout by Myrick et al. (2010) during grow-out; a feed that maintained an adequate growth rate would qualify as suitable for producing market-sized cutthroat trout (20-30 cm TL). Existing salmon and trout diet types are formulations developed for specific purposes in commercial aquaculture, such as maximizing growth or maximizing economic efficiency of growth. Existing salmon and trout diets will be less costly than a new cutthroat-specific diet type because the size of the rainbow trout and salmon market is much larger than the potential cutthroat trout market, and feed companies can benefit from economies of scale.

3. Methods

3.1 Experimental animals

Snake River cutthroat were obtained as eyed embryos from the U.S. Fish and Wildlife Service (USFWS) Jackson National Fish Hatchery, Jackson, Wyoming. Fish were stocked in 200-L tanks, supplied with 14°C flow-through spring water. This temperature was chosen based on the optimal temperature reported for Snake River cutthroat trout by Myrick and Fornshell (2014). First-feeding fry were fed sinking starter crumbles #0 and #1, and were subsequently transitioned to a 2-mm commercial sinking diet (Classic Fry; Skretting North America, Tooele, Utah).

3.2 Diet formulation and production

Experimental diets were formulated to meet or exceed all known nutrient requirements for rainbow trout (NRC, 2011) using ingredients with known palatability (Table 2.1). The experimental formulations were based upon existing commercially available feeds for trout or salmon. Skretting Classic Trout and Skretting Steelhead 3.5 mm floating feeds were used as commercial controls (Skretting North America, Tooele, Utah). Skretting was chosen because previous research on cutthroat trout identified Skretting diets as top performers (Kindschi et al., 2009; Brandt, 2009). The experimental diets were a 3.0-mm commercial type 40:12 (protein: lipid ratio) floating diet, 3.0-mm commercial type 45:16 floating diet, 3.0-mm commercial type 45:24 floating diet, and a 3.0-mm 40:16 floating diet with lysine, methionine and threonine balanced to match that of a 45:16 diet (BFTC experimental diet) (Table 2.1). Floating pellets are accepted by Snake River cutthroat trout as readily as sinking pellets with the added benefit of allowing more accurate monitoring of feed consumption (Ham et al., 2015).

Dry ingredients for each diet were mixed using a horizontal paddle mixer (Marion Mixers, Marion, Iowa) and were ground using an air swept pulverizer (Jacobson 18H, Minneapolis, Minnesota). Lecithin was added and the ingredients were mixed using a horizontal paddle mixer once more. Pellets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) and dried in a pulse-bed drier (OTW-48, Buhler AG, Uzwil Switzerland) for 25 minutes at 102°C with a 10-minute cooling period in order to keep final moisture levels below 10%. Additional oils were added post extrusion by vacuum assisted top-coating (A & J Mixing, Ontario, Canada) in order to produce floating pellets. Experimental floating pellets were measured at approximately 3-mm finished diameter. All feeds were analyzed for nutrient composition following manufacturing (Table 2.2).

3.3 Experimental design

A 6-month feeding trial was conducted at USFWS Bozeman Fish Technology Center (Bozeman, Montana; BFTC). Diets were assigned randomly to five replicate tanks per diet. Snake River cutthroat trout (8.6 g ± 2.0 g; 9.85 ± 0.83 cm TL, mean ± SD) were randomly selected, group weighed, and stocked 20 fish per tank into the 30, 200-L circular fiberglass tanks so that there were no significant differences in initial weight or length across tanks. Fourteen-degree Celsius water was delivered to the tanks at 11 L/min from a partial reuse system, with photoperiod held on a 13-h light and 11-h dark diurnal cycle.

Fish were hand fed a mixture of whole and crumbled pellets twice daily six days per week to apparent satiation for the first month of the experiment. For the remaining five months fish were fed whole pellets using automatic belt feeders (Zeigler Bros. Inc., Gardners, PA) six days per week to mimic the feeding schedule of a production hatchery. Fish were fed at 5% tank biomass for month two, 4% tank biomass for months three and four, and 3% tank biomass for months five and six. These feeding rates consistently resulted in left-over feed within the tanks which were not removed and measured. This practice resulted in conservative estimates of feed consumption rates and feed conversion ratio (FCR).

3.4 Fish sampling

Fourteen fish from the source population were randomly selected at the start of the feed trial and euthanized with 200 mg/L tricaine methanesulfonate (buffered to neutral pH) for determination of whole-body composition. Metrics of growth including individual fish weights, standard lengths, and total lengths were taken at the beginning and end of the study and were additionally used in order to determine specific growth rate (SGR) (Quist et al., 2012) and condition factor (CF) (Neumann et al., 2012).

Monthly fish weights (from batch-weighed tanks) and feed offered were used to determine weight gain and approximate feed conversion ratio (FCR; NRC, 2011). Feed intake per body weight gain (FCR) values were based off of kg of feed offered and not necessarily consumed, hence FCR values were artificially inflated and conservative in nature.

Retail prices of 40-pound (18.1 kg) bags of comparable commercial diet types (as of January 2016) were used to calculate the cost per kg of fish using the following formulae:

$$Cost\ per\ kg\ of\ fish = \frac{cost\ per\ kg\ feed\ (\$) \times feed\ intake\ (dry\ weight\ fed\ (kg))}{body\ weight\ gain\ (wet\ weight\ (kg))}$$

At the end of the 6-month feeding trial, three fish per tank (15 fish per treatment) were randomly selected and euthanized with 200 mg/L tricaine methanesulfonate for determination of whole-body composition. An additional three fish per tank (15 per treatment) were randomly selected, euthanized and dissected to determine visceral somatic index, hepatosomatic index, and muscle ratio.

3.5 Proximate composition analyses

Whole body fish samples, livers, and muscle fillets from each tank were ground for homogeneity and frozen at -20°C. Fish and feed samples were dried to determine moisture content and analyzed in duplicate assays using standard AOAC (1995) methods for proximate composition. Protein was determined using a LECO TRUSPEC nitrogen analyzer (TruspecN, Leco Corporation, St. Joseph, Michigan). Lipid was determined using a Foss Tecator Soxtec HT Solvent Extractor (Model Soxtec HT6 Höganäs, Sweden). Gross energy was determined using isoperibol bomb calorimetry (Parr 1281, Parr Instrument Company Inc., Moline, Illinois). Due to small sample volume, liver samples were only analyzed for moisture and lipid contents.

3.6 Statistical analyses

Differences among response variables within the feed trial were evaluated using JMP® Version 11.2.1 (SAS Institute Inc., Cary, North Carolina). The effect of diet type on the performance variables (i.e., proximate composition, final average weight, percent weight gain, SGR, FCR, survival) were evaluated using one-way ANOVA with the assumption that results were significant with an α level < 0.05. Any p-values below 0.1 were also noted. When the

ANOVA results indicated statistically significant differences, Tukey HSD tests were used for pair-wise comparisons of diet types (Tukey, 1953).

4. Results

4.1 Growth parameters, nutrient utilization and survival rates

The growth performance and nutrient utilization of Snake River cutthroat trout improved when fed diets with higher protein and lipid content than 40:12 ($P< 0.05$; Table 2.3). Trout fed Skretting Steelhead, 45CP:24CL, and BFTC Experimental had significantly higher final weights than trout fed 40CP:12CL ($P<0.05$; Table 2.3). Trout fed Skretting Steelhead and 45CP:24CL also had significantly higher standard lengths than trout fed 40CP:12CL ($P<0.05$; Table 2.3). Trout fed Skretting Steelhead had significantly higher percent gain and SGR than trout fed 40CP:12CL ($P<0.05$). Differences in feed conversion ratio were significant among diets ($P<0.05$), with trends showing Skretting Classic Trout having a higher FCR than both Skretting Steelhead ($P=0.08$) and BFTC Experimental ($P=0.07$) and 40CP:12CL having a higher FCR than BFTC Experimental ($P=0.09$). There were no differences in overall survival ($P>0.05$; Table 2.3).

4.2 Proximate composition and condition indices

At the end of the feeding trial, fish showed some changes in the analyzed parameters compared to those of the initial values (Table 2.4). Although no significant differences were found in protein content of fish fed different diets ($P>0.05$), there were significant differences in moisture content and gross energy ($P<0.05$). Trout fed 40CP:12CL had significantly higher moisture content than fish fed 45CP:24CL, however fish fed 45:24CL had significantly higher gross energy levels than fish fed 40CP:12CL and Skretting Classic Trout ($P>0.05$). While not significant ($P=0.0621$), a trend in lipid content of fish fed different diets was noticeable. No differences were found in viscerosomatic indices, hepatosomatic indices, or muscle ratios

(P>0.05), though trout fed BFTC Experimental had significantly higher condition factors than trout fed Skretting Classic Trout, 40CP:12CL, or 45CP:16CL (P<0.05). There were no significant differences in muscle or liver proximate composition found (P>0.05) (Table 2.5).

4.3 Cost analysis

There were no significant differences found in the estimated cost per kg of fish between the test diets (P>0.05; Figure 2.1).

5. Discussion

This study demonstrated that Snake River cutthroat trout growth performance can be improved by using feeds with an appropriate nutritional profile. Overall, diets greater than 40 percent protein and 12 percent lipid provided the best growth performance for Snake River cutthroat trout. These diets provided higher final weights, lengths, condition factors, and specific growth rates than diets with 40 percent protein and 12 percent lipid. Feed conversion ratios were also lower and proximate composition showed more favorable energy contents for fish fed diets with higher levels of protein and lipids. One of the top performing diets, Skretting Steelhead, is a readily available commercial diet that can be purchased by cutthroat trout culturists.

The two best experimental formulation were the 45CP:24CL and BFTC Experimental. The BFTC Experimental diet, identified as a 40CP:16CL diet amino acid balanced to match the specifications of a 45CP:16CL standard diet, outperformed the 45CP:16CL diet. By supplementing diets with crystalline amino acids, feed manufacturers can reduce the fishmeal inclusion levels. The price of fish meal has remained above US $1100 since it first spiked in 2006, making it one of the most expensive components of feed manufacturing (Indexmundi, 2016). With a lower fishmeal inclusion level, feed production costs can be reduced. This is illustrated in Figure 2.1, wherein a comparable commercial diet to our BFTC Experimental had the lowest cost (USD) per kg of feed of the represented diet formulations in this study ($0.28/kg feed). This contributed to the BFTC Experimental diet having the lowest estimated cost (USD) per kg of fish ($0.61/kg fish). Such an economical and high performing diet would be optimal for fish farming operations and hatcheries and these benefits could be passed along to the consumer. With the exception of the BFTC Experimental diet, diets with a higher cost per kg of feed were associated with a lower cost per kg of fish. For fish farming operations and hatcheries, using a premium fish feed could save money in production costs.

Improving the growth of cutthroat trout during grow-out production would benefit conservation and restoration efforts by potentially improving post-stocking survival. Stocking larger fish would lessen the competitive size advantage that age-0 brook trout have over age-0 cutthroat trout (Behnke, 2002; Peterson et al., 2004). Premium diets such as the 45CP:24CL result in higher energy contents within the fish. Energy reserves are beneficial post-stocking, during which some stocked fish can have significantly emptier stomachs than their wild counterparts for several weeks (Munakata et al., 2000). Mortality during the winter may be due to energy deficits (Coleman and Fausch, 2007), so stocking fish with higher energy stores could also improve their likelihood of survival.

Although we were able to produce market sized fish, Snake River cutthroat trout do not match rainbow trout growth rates or levels of utilization. The highest specific growth rate observed in this study was 0.95%/day (45CP:24CL), which is higher than the specific growth rate (0.72%/day) of juvenile Snake River cutthroat trout found in Ham et al. (2015*b*) that were fed a floating diet. However, rainbow trout can achieve specific growth rates of 1.6%/day or greater (Bullerwell et al., 2016; Craft et al., in press; Wacyk et al., 2012). Feed conversion ratios for the six different diets ranged between 2.15 and 2.93; this is artificially elevated because it was necessary to ensure that excess feed was available as to not limit the overall performance of the diets. These values are higher than the feed conversion ratios of Snake River cutthroat trout fry observed by Kindschi et al. (2009) but similar to those observed in juvenile Snake River cutthroat trout by Ham et al. (2015*b*). In contrast, domesticated rainbow trout can have FCRs of one or better (Craft et al., in press; Burr et al., 2012; Sealey et al., 2011). It is apparent that the cutthroat trout grow-out phase will be longer than that of rainbow trout, leading to additional production costs for culture facilities.

While premium diets did improve the growth performance of Snake River cutthroat trout, the growth performance observed in this study may differ from that of other subspecies. Ham et al. (2015*b*) found differences between Snake River and westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) growth rates at optimal temperatures despite being fed the same nutrient-dense diet. This is not unexpected as different salmonids have been reported to utilize the same feed ingredients with varying efficiency (Azevedo et al., 2004; Krogdahl et al., 2004), though comparisons are generally made between more distantly-related species.

Diets with protein: lipid ratios greater than 40:12 are produced by commercial feed manufacturers. While grow-out of Snake River cutthroat trout was improved, it is possible that a more species-specific diet could provide better growth performance. Diets within this study were formulated to meet or exceed the nutrient requirements of rainbow trout (NRC, 2011), and these nutrient requirements could differ from that of cutthroat trout. Additional nutrition research such as determining the lysine requirement, optimal vitamin and mineral mixture concentration, and optimal digestible protein to digestible energy ratio for juvenile cutthroat trout would help determine the optimal diet for cutthroat trout. If the nutrient requirements for cutthroat trout substantially differ from that of rainbow trout, then cutthroat trout-specific diets may be justified and necessary to optimize production.

Another avenue of cutthroat trout research that has yet to be explored is genetic selection. Rainbow trout have been genetically selected for faster growth (Hulata, 2001) and to utilize plant based diets efficiently (Overturf et al., 2013), yet genetic selection has not been utilized in cutthroat trout culture. Anecdotal evidence suggests that there are strain specific differences in growth and feeding behavior in cutthroat trout, so research is necessary to determine which, if any, strains grow most efficiently and to identify the specific genetic differences that could cause these differences. Selecting fast-growing individuals could markedly improve the growth rates observed in cutthroat trout culture systems.

6. Conclusions

Floating diets containing greater than 40 percent protein and 12 percent lipid provide the greatest growth performance in Snake River cutthroat trout during grow-out to market size (20-30 cm TL). In particular, amino acid balanced diets provide a cost efficient diet choice that performs as well or better than their standard counterparts. Diets with 45 percent protein and 24 percent lipid provide the highest energy content within fish, providing the fish with greater post-release energy reserves. All diets included within this study were formulated to the nutrient requirements of rainbow trout, so further research is necessary to determine if these requirements fit cutthroat trout. Additional research into genetic selection of cutthroat trout may also improve growth and diet utilization of hatchery reared fish.

Tables and Figures

Table 2.1. Composition of the experimental diets fed to juvenile Snake River Cutthroat trout (*Oncorhynchus clarkii behnkei*) in laboratory feeding trial conducted at Bozeman Fish Technology Center in 2014-2015.

Ingredients[1] (%-as fed)	Diet 40:12	Diet 45:16	Diet 45:24	BFTC Diet
Corn Protein Concentrate	3.00	3.00	3.00	3.00
SC Blood 8521	3.00	3.00	3.00	3.00
Soybean Meal 48%CP	15.00	15.00	15.00	15.00
Feather Meal	6.50	6.50	6.50	6.50
Chicken 42-ADF	6.00	9.00	9.00	6.00
Menhaden Fish Meal Special Select	17.00	23.00	23.00	17.00
Wheat Flour	33.44	22.74	13.60	21.15
Wheat Gluten Meal	0.00	0.00	1.00	1.00
Menhaden Fish Oil	4.50	6.00	10.00	8.50
Lecithin	2.00	2.00	2.00	2.00
Poultry Fat	4.50	6.00	10.00	8.50
Stay-C 35	0.20	0.20	0.20	0.20
Vitamin premix ARS[2]	1.00	1.00	1.00	1.00
TM ARS 640[3]	0.10	0.10	0.10	0.10
Astaxanthin	0.00	0.00	0.04	0.04
Grobiotic A	0.00	0.00	0.10	0.10
Monocalcium Phosphate	2.00	0.80	0.80	2.00
Choline Cl 50%	1.00	1.00	1.00	1.00
Magnesium Oxide	0.06	0.06	0.06	0.06
DL-Methionine	0.70	0.60	0.60	0.70
Lysine HCl	0.00	0.00	0.00	2.55
Threonine	0.00	0.00	0.00	0.60

[1] Ingredients were sourced from commercial fish feed manufacturers (Skretting, Tooele, Utah and Rangen, Buhl, Idaho).
[2] Contributed per kg of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D3, 2160 IU; vitamin E (as DL-%-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 2.2. Analyzed composition of feeds (% dry matter) for Snake River cutthroat *Oncorhynchus clarkii behnkei* fed in laboratory feeding trials conducted at Bozeman Fish Technology Center in 2014-2015.

Parameters	Experimental Diets					
	Skretting Classic Trout	Skretting Steelhead	40 CP: 12 CL	45 CP: 16 CL	45 CP: 24 CL	BFTC Experimental
Moisture	5.3 ± 0.2	5.3 ± 0.2	5.6 ± 0.2	2.8 ± 0.0	3.0 ± 0.2	3.6 ± 0.0
Protein	40.0 ± 0.1	44.8 ± 0.1	37.3 ± 0.1	43.1 ± 0.0	42.0 ± 0.1	42.1 ± 0.1
Lipid	12.0 ± 0.1	15.0 ± 0.1	11.8 ± 0.3	16.4 ± 0.1	23.1 ± 0.2	19.4 ± 0.2
Gross Energy (cal g^{-1})	4457 ± 16	4641 ± 25	4533 ± 6	4974 ± 13	5319 ± 14	5094 ± 53

Table 2.3. Growth parameters and nutrient utilization of juvenile Snake River Cutthroat trout *Oncorhynchus clarkii behnkei* fed in laboratory feeding trials conducted at Bozeman Fish Technology Center in 2014-2015. Values are means ± SEM of quintuplicate groups within a column. Means with different superscript letters are significantly different (P < 0.05). Means having the same superscript letters were not significantly different. The absence of letters indicates no significant difference between treatments found in ANOVA.

Parameters	Experimental Diets					
	Skretting Classic Trout	Skretting Steelhead	40 CP: 12 CL	45 CP: 16 CL	45 CP: 24 CL	BFTC Experimental
Initial weight (g)	8.6 ± 0.2	8.7 ± 0.3	8.5 ± 0.3	8.8 ± 0.2	8.6 ± 0.2	8.7 ± 0.3
Final weight (g)	37.9 ± 9.6^{ab}	47.3 ±4.7^{a}	34.7 ± 3.9^{b}	41.9 ± 3.5^{ab}	46.4 ± 5.3^{a}	46.5 ± 3.5^{a}
Initial SL[1]	8.6 ± 0.1	8.6 ± 0.1	8.5 ± 0.1	8.6 ± 0.0	8.7 ± 0.1	8.6 ± 0.1
Final SL[2]	13.7 ± 1.1^{ab}	14.7 ± 0.5^{a}	13.4 ± 0.4^{b}	14.2 ± 0.6^{ab}	14.6 ± 0.6^{a}	14.2 ± 0.3^{ab}
Percent weight gain	343 ± 126^{ab}	460 ± 43.7^{a}	312 ± 51.2^{b}	397 ± 50.1^{ab}	445 ± 57.7^{ab}	437 ± 37.6^{ab}
SGR[3]	0.80 ± 0.15^{ab}	0.95 ± 0.04^{a}	0.78 ± 0.07^{b}	0.88 ± 0.05^{ab}	0.93 ± 0.06^{ab}	0.93 ± 0.04^{ab}
FCR[4]	2.93 ± 0.87^{a}	2.16 ± 0.22^{a}	2.89 ± 0.29^{a}	2.30 ± 0.28^{a}	2.25 ± 0.26^{a}	2.15 ± 0.16^{a}
Percent survival	91 ± 13	95 ± 5	91 ± 6	98 ± 3	95 ± 6	93 ± 11

[1]Initial SL = initial standard length (cm).

[2]Final SL = final standard length (cm).

[3]SGR = specific growth rate (%$\cdot$ day^{-1}).

[4]FCR = feed conversion ratio.

Table 2.4. Whole body proximate composition (% dry matter) and condition indices of juvenile Snake River Cutthroat trout *Oncorhynchus clarkii behnkei* fed in laboratory feeding trials conducted at Bozeman Fish Technology Center in 2014-2015. Values are means ± SEM of quintuplicate groups within a column. Means with different superscript letters are significantly different (P < 0.05). Means having the same superscript letters were not significantly different. The absence of letters indicates no significant difference between treatments.

Parameter	Initial *	Experimental Diets					
		Skretting Classic Trout	Skretting Steelhead	40 CP:12 CL	45 CP:16 CL	45 CP:24 CL	BFTC Experimental
Moisture	70.5	71.2 ± 0.6^{ab}	69.8 ± 1.4^{ab}	71.6 ± 1.2^{a}	70.4 ± 1.6^{ab}	69.0 ± 1.2^{b}	69.9 ± 1.7^{ab}
Protein	20.4	18.4 ± 0.1	18.6 ± 0.5	18.0 ± 0.4	18.7 ± 0.9	18.6 ± 0.6	18.4 ± 0.9
Lipid	6.6	6.6 ± 0.9	7.3 ± 1.5	6.6 ± 0.9	7.6 ± 1.1	9.0 ± 1.3	8.0 ± 1.9
Gross Energy[1]	1786	1637 ± 69^{b}	1690 ± 90^{ab}	1642 ± 67^{b}	1800 ± 126^{ab}	1895 ± 126^{a}	1801 ± 162^{ab}
VSI[2]	—	8.9 ± 0.9	8.8 ± 0.9	9.0 ± 1.5	9.4 ± 1.0	9.2 ± 0.7	8.7 ± 1.4
HSI[3]	—	0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
Muscle ratio	—	42.4 ± 3.0	44.0 ± 2.7	42.8 ± 1.8	43.3 ± 2.9	43.3 ± 3.2	41.7 ± 1.6
Condition factor	—	1.33 ± 0.04^{b}	1.38 ± 0.03^{ab}	1.36 ± 0.03^{b}	1.36 ± 0.05^{b}	1.37 ± 0.03^{ab}	1.43 ± 0.03^{a}

[*] Initial whole body composition values not used in statistical analyses.
[1] Units= (cal g^{-1}).
[2] VSI= viserasomatic index
[3] HSI= hepatosomatic index

Table 2.5. Muscle and liver proximate composition (% dry matter) of juvenile Snake River Cutthroat trout *Oncorhynchus clarkii behnkei* fed in laboratory feeding trials conducted at Bozeman Fish Technology Center in 2014-2015. Values are means ± SEM of quintuplicate groups within a column. The absence of superscript letters indicates no significant difference between treatments.

Source	Parameters	Experimental Diets					
		Skretting Classic Trout	Skretting Steelhead	40 CP: 12 CL	45 CP: 16 CL	45 CP: 24 CL	BFTC Experimental
Muscle	Moisture	78.0 ± 0.5	77.7 ± 0.3	77.6 ± 0.4	77.7 ± 0.6	77.3 ± 0.8	77.4 ± 1.2
	Protein	20.0 ± 0.4	19.5 ± 07	19.5 ± 0.4	18.9 ±1.4	19.4 ± 0.8	19.3 ± 1.8
	Lipid	0.9 ± 0.3	1.2 ± 0.3	1.5 ± 0.9	1.4 ± 0.4	2.0 ± 1.1	1.5 ± 0.5
	Gross Energy[1]	1290 ± 118	1289 ± 54	1265 ± 43	1243 ± 60	1367 ± 106	1327 ± 71
Liver	Moisture	74.6 ± 1.1	75.0 ± 2.3	73.7 ± 1.0	73.2 ± 2.2	72.9 ± 1.2	73.3 ± 2.1
	Lipid	2.6 ± 0.8	2.6 ± 0.8	2.1 ± 0.8	2.6 ± 1.5	3.1 ± 1.1	3.3 ± 1.2

[1]Units= (cal g^{-1}).

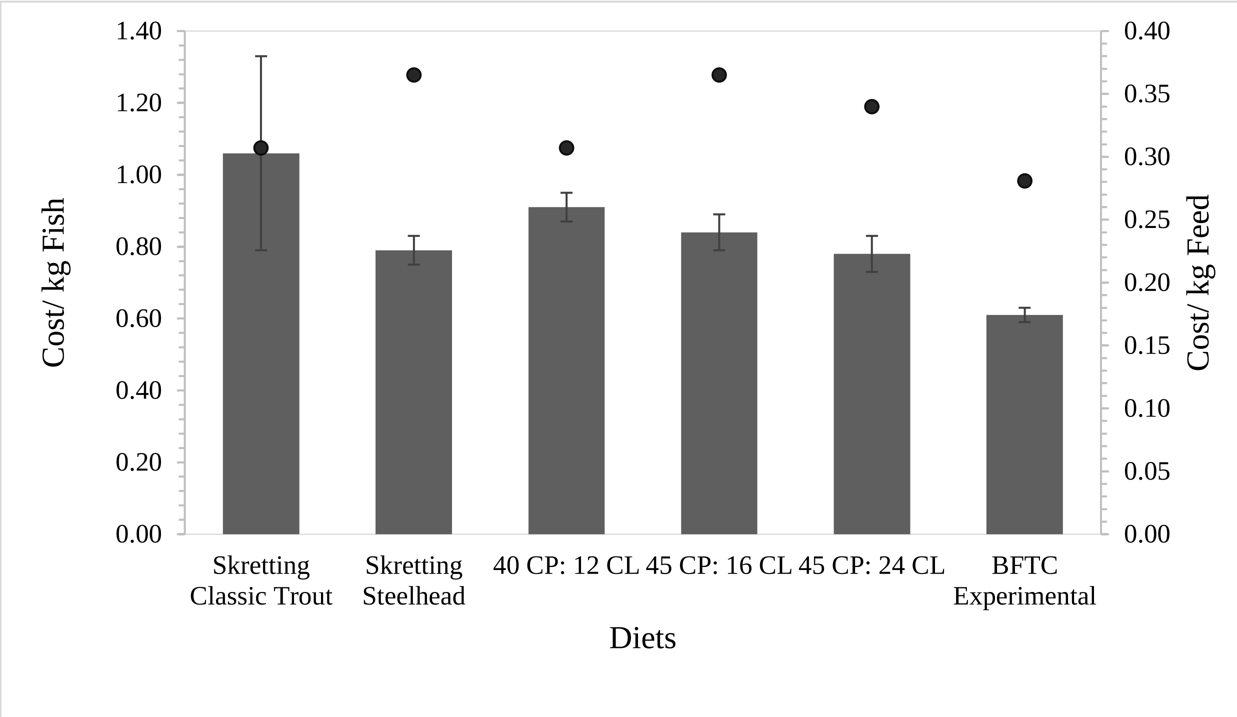

Figure 2.1. Estimated cost (USD) per kg of fish (columns) and estimated cost (USD) per kg of feed (points) of juvenile Snake River Cutthroat trout *Oncorhynchus clarkii behnkei* fed in laboratory feeding trials conducted at Bozeman Fish Technology Center in 2014-2015. Column values are means ± SEM of quintuplicate groups within a column. The absence of letters indicates no significant difference between treatments.

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, 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, 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., 2002. Trout and salmon of North America. The Free Press, New York, USA.

Behnke R.J., 1992. Native trout of western North America. American Fisheries Society 429 monograph no. 6, USA.

Brandt, M.M., 2009. Optimal starter diets and culture conditions for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). M.S. Thesis. Colorado State University, Fort Collins, CO.

Bosakowski, T., Wagner, E.J., 1994*a*. A survey of trout fin erosion, water quality, and rearing conditions at state fish hatcheries in Utah. Journal of the World Aquaculture Society 25, 308– 316.

Bosakowski, T., Wagner, E.J., 1994*b*. Assessment of fin erosion by comparison of relative fin length in hatchery and wild trout in Utah. Canadian Journal of Fisheries and Aquatic Sciences 51, 636– 641.

Bullerwell, C.N., Collins, S.A., Lall, S.P., and Anderson, D.M., 2016. Growth performance, proximate and histological analysis of rainbow trout fed diets containing *Camelina sativa* seeds, meal (high-oil and solvent-extracted) and oil. Aquaculture 452, 342– 350.

Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture, 334, 110– 116.

Coleman, M.A., Fausch, K.D., 2007. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River cutthroat trout reared in laboratory streams. Transactions of the American Fisheries Society 136, 639– 654.

Craft, C.D., Ross, C., Sealey, W.M., Gaylord, T.G., Barrows, F.T., Fornshell, G., Myrick, C.A. in press. Growth, proximate composition, and sensory characteristics of rainbow trout *Oncorhynchus mykiss* consuming alternative proteins. Aquaculture.

Ersbak, K., Haase, B.L., 2011. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3 (2), 142– 151.

Ham, B.R., Barrows, F.T., Huttinger, A., Duff, G.C., Yeoman, C.J., Maskill, M.G., Sealey, W.M., 2015*a*. Evaluation of dietary soy sensitivity in Snake River cutthroat trout. North American Journal of Aquaculture 77 (2), 195– 2015

Ham, B.R., Myrick, C.A., Barrows, F.T., Yeoman, C. J., Duff, G.C., Maskill, M.G., Sealey, W.M., 2015*b*. Feed characteristics alter growth efficiency in cutthroat trout. Journal of Fish and Wildlife Management 6 (1), 83– 91.

Hohenlohe, P.A., Amish, S.J., Catchen, J.M., Allendorf, F.W., Luikart, G., 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources 11, 117–122.

Hulata, G., 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111, 155– 173.

Indexmundi, 2016. Fishmeal commodity price. http://www.indexmundi.com/commodities/?commodity=fish-meal&months=120. Accessed: 1 April, 2016.

JMP®, Version 11.2.1. SAS Institute Inc., Cary, NC, 1989– 2007.

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. Progressive Fish–Culturist 56 (1), 13 – 18.

Krogdahl, A., Sundby, A., Olli, J.J., 2004. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. Aquaculture 229 (1), 325– 360.

Metcalf, J.L., Pritchard, V.L., Silverstri, S.M., Jenkins, J.B., Wood, J.S., Cowley, D.E., Evans, R.P., Shiozawa, D.E., Martin, A.P., 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology 16, 4445– 4454.

Munakata, A., Björnsson, B.T., Jönsson, E., Amano, M., Ikuta, K., Kitamura, S., Kurokawa, T., Aida, K., 2000. Post-release adaptation processes of hatchery-reared honmasu salmon parr. Journal of Fish Biology 56,163– 172.

Myrick, C.A., Fornshell, G., 2014. Early rearing of cutthroat trout. Western Regional Aquaculture Center, Seattle, Washington, USA.

Myrick, C.A., Kindschi, G. Fornshell, G., Cline, K., Nelson, C., Seals, J., Webb, M., Kappenman, K., 2010. Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout. Western Regional Aquaculture Center, Seattle, Washington, USA.

National Research Council, 2011. Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Neumann, R.M., Guy, C.S., Willis, D.W., 2012. Length, weight, and associated indices. Chapter 14 In Zale, A.V., Parrish, D.L., Sutton, T.M. (eds). Fisheries techniques third edition, American Fisheries Society, Bethesda, Maryland, USA.

Overturf, K., Barrows, F.T., Hardy, R.W., 2013. Effect and interaction of rainbow trout strain (*Oncorhynchus mykiss*) and diet type on growth and nutrient retention. Aquaculture Research 44, 604– 611.

Piper R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J.R., 1982. Fish hatchery management. Washington, DC, U.S. Department of Interior, Fish & Wildlife Service.

Peterson, D.P., Fausch, K.D., White, G.C., 2004. Population ecology of an invasion: effects of nonnative brook trout on native cutthroat trout. Ecological Applications 14, 754–772.

Quist, M.C., Pegg, M.A., DeVries, D.R., 2012. Age and growth. Chapter 15 In Zale, A.V., Parrish, D.L., Sutton, T.M. (eds). Fisheries techniques third edition, American Fisheries Society, Bethesda, Maryland, USA.

Sealey, W.M., Hardy, R.W., Barrows, F.T., Pan, Q., Stone, D.A.J., 2011. Evaluation of 100% fish meal substitution with chicken concentrate, protein poultry by-product blend, and chicken and egg concentrate on growth and disease resistance of juvenile rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society, 42, 46–55

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, 61– 69.

Tukey, J., 1953. The problem of multiple comparisons. Princeton University, Princeton, New Jersey, USA.

Underwood, Z.E., Myrick, C.A., Rogers, K.B., 2012. Effect of acclimation temperature on the upper thermal tolerance of Colorado River cutthroat trout *Oncorhynchus clarkii pleuriticus* (Richardson). Journal of Fish Biology 80 (7), 2420-2433.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, The American Institute of Fishery Research Biologist, and the American Society of Ichthyologists and Herpetologists), 2004. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland, USA.

Wacyk, J., Powell, M., Rodnick, K., Overturf, K., Hill, R.A., Hardy, R., 2012. Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture, 356, 223– 234.

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, 173– 187.

Webster, C.D., Lim, C.E. (eds.), 2002. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, New York, USA.

CHAPTER 3: EVALUATION OF SUBADULT SNAKE RIVER CUTTHROAT TROUT: DOES DIET OR SIZE INFLUENCE BEHAVIOR?

1. Summary

Snake River cutthroat trout (*Oncorhynchus clarkii behnkei*) were exposed to open field testing and a model predator (great blue heron; *Ardea herodias*) to determine if there were differences in behavior relating to diet or size. Fish were fed one of two commercially available control diets (Skretting Classic Trout and Skretting Steelhead) or four experimental diets formulated with varying levels of crude protein (CP) and crude lipid (CL) (40CP:12CL, 45CP:16CL, 45CP:24CL, 40CP:16CL amino acid balanced to equal 45CP:16CL [BFTC Experimental]) for six months prior to behavioral observations and were divided into one of two different size classes (small: 12 ± 2.5 cm TL; large: 20 ± 2.5 cm TL). Testing was repeated on a medium size class (16 ± 2.5 cm TL). The results showed that small fish were less likely to freeze than large fish during open field testing and were potentially more likely to dart than large fish during simulated predator attacks. Differences in freezing response between small and large fish fed different diets and possible differences in darting response between medium fish fed different diets were observed. These results suggest that diet formulation can potentially alter the prevalence of cutthroat trout anti-predator behavior, and differences in behaviors can exist between fish of differing size classes. The presence of anti-predator responses in hatchery-reared cutthroat is encouraging for ongoing restoration efforts.

2. Introduction

Increased emphasis has been placed on the propagation of native salmonids to restore native populations and as an alternative to non-native sport fish (Ham et al., 2015). As such it is important that stocked cutthroat trout are capable of surviving post-release in the face of long-term challenges such as predators. However, past studies have indicated that there is a dramatic level of mortality with newly released hatchery salmonids (Brown and Laland, 2001); for example, less than five percent of hatchery reared Pacific salmon survive to adulthood (McNeil, 1991). Major causes of mortality for hatchery-reared salmonids include predation (Biro et al., 2004) and nutritional deprivation (Ersbak and Haase, 2011). This trend of low survival has been previously reported to hold true for hatchery reared cutthroat trout as well (Miller, 1954).

There is a growing body of research suggesting that domesticated strains of salmonids are generally bolder and more aggressive than their wild counterparts (Conrad et al., 2011). This behavior type, correlated with higher growth rates (Biro and Post, 2008), may not be the most advantageous for post-stocking survival. Bigger, bolder individuals tend to engage in risky behavior to support their elevated appetites (Alvarez and Nicieza, 2003), making them more susceptible to predation. Biro et al. (2004) found that domestic rainbow trout took greater risks while foraging than wild-type individuals, resulting in greater growth rates. In replicated lake experiments, it was found that the higher-risk foraging behavior conferred greater survival than the more risk-averse behavior shown by wild-type individuals when avian predation was low, but that the reverse was true when avian predation was high.

Alvarez and Nicieza (2003) provided evidence that anti-predator behavior is highly sensitive to artificial rearing. They found that both first- and second-generation hatchery-reared juvenile brown trout (*Salmo trutta*) were insensitive to the presence of a piscivorous adult brown trout, while wild-caught juveniles utilized shelter and decreased daytime activity in the presence of the predator. Interestingly, Biro et al. (2004) found that domestic rainbow trout were capable of showing appropriate anti-predator behavior in the presence of an avian predator, despite no prior experience.

While some captive-reared salmonids may lose their anti-predator behaviors, it may be possible to condition predator-naïve hatchery fish to recognize predator risk. Brown and Smith (1997) found that juvenile rainbow trout significantly increased anti-predator behaviors, such as freezing and seeking cover, when exposed with conspecific skin extracts. These alarm cues can be used to condition trout to recognize a predator (Brown and Smith 1998).

Diet has also been shown to influence bold and exploratory behavior in fish. Holley et al. (2014) found that zebrafish (*Danio rerio*) became more exploratory when maintained on a diet of brine shrimp with a predictable delivery schedule than a variable delivery schedule. However, a high (3.5 mL *Artemia* nauplii/week) or low (0.92 mL *Artemia* nauplii /week) diet ration had no effect on behavior. Interestingly, Chapman et al. (2010) found that unpredictability in food supply in early life resulted in bolder and more exploratory Trinidadian guppies (*Poecilia reticulata*). Borcherding and Magnhagen (2008) suggested that malnourished juvenile European perch (*Perca fluviatilis*) were bolder than their well-nourished counterparts. Similar findings have been reported for Atlantic salmon (*Salmo salar*) (Vehanen, 2003) and Crucian carp (*Carassius carassius*) (Pettersson and Brönmark, 1993). To combat the effects of malnourishment, individuals may raise their activity and exhibit more risky behavior in order to increase the chance of encountering food (Brown et al., 2006; Lima, 1998).

Diet can also influence the success of training trout to recognize a novel predator. Brown et al. (2011) found that juvenile rainbow trout that were fed a low food ration (1% mean body mass) retained recognition of a novel predator longer than trout fed on a high food ration (5% mean body mass). Although studies of the influence of food ration and predictability on boldness and predator recognition exist, there are no current studies looking at whether diet formulation has an impact on fish behavior. There is also a lack of behavioral studies conducted on cutthroat trout. Understanding the behaviors of hatchery-reared cutthroat would provide insight into the potential effects of hatchery propagation on innate behaviors.

With predation playing a major role in salmonid post-stocking survival (Biro et al., 2004), knowing whether hatchery-reared cutthroat trout display anti-predator behavior could improve the success of conservation efforts of cutthroat trout. The purpose of this study was to document behavior of Snake River cutthroat trout in a novel environment and when exposed to a novel predator. Experimental animals underwent an open field test, a standard method to measure bold/shy and/or exploratory/avoidance behaviors (Conrad et al., 2011), and a predator exposure to a model great blue heron (*Ardea herodias*). Great blue herons readily consume hatchery-reared trout (Glahn et al., 1999) and are common throughout the United States. Snake River cutthroat trout of different sizes from the six diet treatments were used to explore the relationship between behavior and size and/or diet type.

3. Methods

3.1 Experimental animals

Snake River cutthroat were obtained as eyed embryos from the U.S. Fish and Wildlife Service (USFWS) Jackson National Fish Hatchery, Jackson, Wyoming. Fish were stocked in 200-L tanks, supplied with 14°C flow-through spring water and fed sinking starter crumbles #0 and #1, and progressed to a 2-mm commercial sinking diet (Classic Fry; Skretting North America, Tooele, Utah). Once individuals were roughly nine grams in size they were stocked 20 fish per tank into 30, 200-L circular fiberglass tanks and fed one of six floating diets (See Chapter 2) for a six-month period. Fourteen-degree Celsius water was delivered to the tanks at

11 L/min from a partial reuse system, with photoperiod held on a 13-h light and 11-h dark diurnal cycle.

During the behavior trials, individual fish were stocked into six 300-L raceways (2.4 m L × 0.6 m W × 0.4 m H) receiving 6 L/min of 14°C spring water. Submersible pumps were placed at a fixed off-center position on the upstream end of the raceway to create a gentle circulating current. Raceways inlets and standpipes were blocked from access by the fish with metal screens. Once each round of behavioral observations was collected, tanks were drained and refilled prior to the next round of observations in order to lessen the possibility of residual alarm cues.

3.2 Experimental design

Twenty-four hour behavior trials were conducted at the USFWS Bozeman Fish Technology Center (Bozeman, Montana). A 2 × 6 factorial design was used with two different size classes (small (12 ± 2.5 cm TL), large (20 ± 2.5 cm TL)) and six diets. The diets included two commercial controls (3.5-mm Skretting Classic Trout and Skretting Steelhead floating pellet) and four experimental diets (3.0-mm commercial type 40:12 (protein: lipid ratio) floating diet, 3.0-mm commercial type 45:16 floating diet, 3.0-mm commercial type 45:24 floating diet, and a 3.0-mm 40:16 floating diet with the amino acid balance of a 45:16 diet (BFTC experimental diet)) that fish were maintained on for approximately six months (See Chapter 2). Water temperature was maintained at 14°C and photoperiod was 13-h light and 11-h dark diurnal cycle. A total of 126 fish were used for the behavioral trials, with n=42 for each size group, n=14 for each diet, and n=7 for each size/diet combination. The six raceways were utilized in such a way that one replicate of each of the twelve treatment combinations was used within a forty-eight hour period.

A replicate tank from each diet was randomly chosen and the largest and smallest fish within the tank were selected based on visual inspection and placed within an opaque bucket for transfer to one of the six experimental raceways. The bottom of the raceway was divided into 20 cm × 56 cm segments in order to track movement of the fish along the length of the tank, with each segment length slightly greater than the average total length of all fish at the end of the six month feeding trial. Raceways were surrounded by opaque shields to reduce outside disturbance (Figure 3.1).

Individual fish were placed within a 16-cm diameter PVC pipe section placed in the center of the far upstream end of the raceway and allowed to settle for 2 minutes prior to the start of the open field tests. The PVC segment was then removed and behaviors of the fish were recorded for 8 minutes using a GoPro Hero 3+ HD video camera (Go Pro, USA). No water current was present during the open field tests; however pumps were activated upon the completion of recording.

Fish were placed in the predator exposure tanks for approximately 18 hours before the start of the predator exposure (Figure 3.1). The GoPro camera was positioned above the tank midline and the predator was fixed to the upstream end of the tank. The fish were allowed to settle from any disturbance related to camera and predator placement for 10 minutes prior to the start of the exposure. Individuals were recorded for 5 minutes before the model avian predator was activated, striking the water every 30 seconds for a 2.5 minutes (5 strikes). The predator was then withdrawn, and the individual fish was recorded for an additional 5 minutes. At the end of the predator exposure each individual was euthanized using tricaine methanesulfonate (200 mg/L, buffered to neutral pH), weighed (nearest g), and measured for standard and total length (mm). Individuals were dissected in order to determine maturity and sex. The study was later repeated with randomly selected medium sized fish (16 ± 2.5 cm TL, n=42) from randomly selected tanks fed each diet.

3.3 Behavioral analyses

Video recordings were later viewed using Windows Media Player (Microsoft, USA) and manually scored for behaviors expected to reflect variation in either exploration or anti-predator behaviors. For the open field tests, behaviors of interest included the presence or absence of freezing (no visible body/fin movement), thigmotaxis (wall hugging, within 5 cm of the tank wall), maintaining position (visible body/fin movement without net horizontal movement within the tank), and swimming (visible body/fin movement with net horizontal movement within the tank). Rate of exploration (# lines crossed/time) was also measured and whether the fish crossed the midline of the tank or returned to the starting zone was noted. For the predator exposures, behaviors of interest included the presence or absence of darting (brief and sudden accelerated swimming that can be characterized as a C-start), thigmotaxis, freezing, swimming, and maintaining position. Presence/absence of darting or freezing in response to each heron strike was noted. While durations of behaviors were also recorded, statistical analyses were not performed on durations due to violations in normality and homogeneity of variance.

3.4 Statistical analyses

Statistical analyses were conducted in R language for statistical computing (v. 3.2.4 Revised; R Core Team, 2016) within the RStudio environment (v. 0.99.893; RStudio, 2015). Separate statistical analyses were conducted on small/large and medium trials. Global models were initially used for analyses and covariates (sex and maturity) were removed from final models due to insignificance ($P>0.05$). Probabilities of darting, freezing, thigmotaxis, swimming, maintaining position, crossing tank midline, and returning to starting block were modeled within separate general linear models with binomial distribution (logit link), which included all predictor variables (class and/or diet) (R Core Team, 2016). If there was evidence of complete or quasi-complete separation, the model was run using Firth bias-adjusted estimates using JMP® Version 11.2.1 (SAS Institute Inc., Cary, North Carolina). Total counts of freeze and dart responses were modeled within separate general linear models with quasipoisson distribution (log link) due to over-dispersion, which included all predictor variables (class and/or diet) (R Core Team, 2016). To compare behaviors between multiple heron strikes or between time periods within the predator exposure, mixed general linear models with binomial distribution (logit link) was used with time as the categorical predictor variable, package *lme4* (Bates et al., 2015). The arcsine transformation was used to compare exploration rate among predictor variables. Results from both the open field test and predator exposure were analyzed using type three ANOVA, package *car* (Fox and Weisberg, 2011), and Tukey-adjusted pairwise comparisons were performed using package *lsmeans* (Lenth, 2016). In the event that no significant pairwise comparisons were found using the Tukey-adjustment, unadjusted multiple comparisons were run in order to determine avenues of further investigation. Tables and figures report only Tukey-adjusted pairwise comparisons.

4. Results

4.1 Open field tests

In general, Snake River cutthroat trout were likely to show all measured behavioral responses. Overall, small and large fish had the greatest probability of swimming (0.93) and thigmotaxis (0.99), followed by maintaining position (0.84) and freezing (0.53; Table 3.1). Similar probabilities of swimming (0.95) and thigmotaxis (1.00) were found in medium fish, along with a slightly higher probability of maintaining position (0.90) and freezing (0.67; Table

3.2). Small and large fish had a probability of 0.73 for crossing the tank midline and returning to the starting block, with an average exploration rate of 0.27 lines crossed·s^{-1}. Medium fish had a slightly higher probability (0.81) of crossing the tank midline and returning to the starting block, with a similar average exploration rate of 0.31 lines crossed·s^{-1}. Large fish had a significantly higher probability of freezing than small fish (P<0.05).There were no other significant differences in the probability of behaviors occurring in relation to diet or sex (P>0.05).

4.2 Predator exposures

Cutthroat trout had a significantly higher probability of freezing or darting during the exposure than before or after the exposure (Tables 3.3 and 3.4; P<0.05). Fish had similar probabilities of thigmotaxis throughout the trial duration and did not significantly differ in their probabilities of maintaining position or swimming pre- or post-exposure, however there was a trend in reduced swimming of small and large fish (P=0.0636). There were no significant differences in probabilities of fish freezing by heron strike (Figures 3.2 and 3.3; P>0.05), but the probability of darting is significantly higher during the first strike than the last three strikes (Figure 3.2; P<0.05) in small and large fish. This was not observed in medium fish (Figure 3.3).

The small and large cutthroat trout darted in response to the five heron strikes an average of 1.07 times and froze an average of 2.04 times (Table 3.5), while the medium individuals darted an average of 0.64 times and froze an average of 2.64 times (Table 3.6). Overall, fish had a higher probability of freezing in response to heron strikes than darting. There were no significant differences in the total darting responses and freezing responses by diet or class (P>0.05), however a size class trend (P=0.0652) and diet (medium fish only) trend (P=0.0825) was evident in darting probability. Significant differences in freezing probability were found in small and large fish by diet (P<0.05). While no significant differences were found using Tukey's multiple comparisons, 40CP:12CL (0.93) and BFTC Experimental (0.93) were found to have significantly higher probabilities of freezing than Skretting Steelhead (0.50) using unadjusted multiple comparisons. The probability of freezing was not impacted by diet in medium fish (P>0.05).

5. Discussion

This study is the first to assess the behavioral responses of hatchery-reared Snake River cutthroat trout, and the first to assess the impacts of diet type on the anti-predator responses of fish. We demonstrated the potential for differences in both darting and freezing response by diet type and darting responses by size class. Overall, the Jackson National Fish Hatchery strain of Snake River cutthroat trout exhibited predator avoidance behavior, suggesting that the hatchery environment has not eliminated their responses to perceived threats.

Small Snake River cutthroat trout exhibited a lower probability of freezing than large-sized individuals in open field testing which was not evident during predator exposures. However, small fish were more likely to dart in response to a heron strike than large-sized fish, suggesting that response to predation risk may be size-dependent. Although this difference was not statistically significant, further investigation is warranted to determine if size-dependent anti-predator behavior exists in fish. Such differences have previously been reported in Iberian rock lizards (*Iberolacerta monticola*) (Martín and López, 2003), American lobster (*Homarus americanus*) (Wahle, 199), and the adzuki bean beetle (*Callosobruchus chinensis*) (Hozumi and Miyatake, 2005). The decision to escape a predator via darting has costs, such as energy expenditure and lost opportunities to engage in other behaviors, so the probability of a darting response should reflect the animal's assessment of the risk of imminent capture and the probability of successful evasion (Lima and Dill, 1989). Social interactions in small groups of salmonids can lead to the formation of dominance hierarchies, resulting in dominants gaining larger shares of available food and exhibiting aggression toward subordinates, causing higher cortisol levels in these fish (Gilmour et al., 2005). With elevated cortisol levels in subordinate fish it is possible that these smaller fish will exhibit a more severe response to risk than larger fish, which may explain the higher prevalence of darting in small fish. However, this is not to say that small fish are more cautious than larger fish; Snake River cutthroat trout exhibited consistently high levels of thigmotaxis in both the open field tests and predator exposures and all size classes exhibited freezing behavior during both tests.

Differences in freezing probability by diet type were observed in small and large fish, while a trend in differences in darting probability by diet type was observed in medium fish. There's evidence that small and large fish fed BFTC Experimental and 45CP:16CL have higher probabilities of freezing than fish fed Skretting Steelhead and additional evidence of a diminished darting response in medium fish fed Skretting Classic Trout. These findings warrant further investigation to determine if diet type can impact the anti-predator behavior of fish. The BFTC Experimental, 45CP:16CL, and Skretting Steelhead are all formulated to meet the same protein and lipid ratio, and have similar gross energy contents (see Chapter 2). Both Skretting diets have proprietary formulations, so it remains unclear whether the diets differ substantially in ingredients or inclusion levels. All diet formulations are designed to have a balanced amino acid profile, though these do differ slightly depending on the use of the ideal protein concept or conventional growth response assays in determining essential amino acid requirements. While deficiencies in essential amino acids are known to impact growth and feeding behavior (NRC, 2011), there is no evidence that such deficiencies can impact other behaviors. There are also various fat and oil sources that can contribute to the lipid content of fish feeds, however deficiencies in essential fatty acids result in various pathologies. Deficiencies in DHA has been found to prevent schooling behavior in yellowtail (*Seriola quinqueradiata*) larvae (Ishizaki et al., 2001) and deficiencies n-3 HUFA and DHA reduces the burst swimming response to visual stimuli in larval gilthead seabream (*Sparus aurata*) (Benítez-Santana et al., 2007). Whether similar effects would be present in cutthroat trout is unknown, and would be worthy of further research.

There has been some debate on whether open field tests and predator exposures are both acceptable tests of boldness, or whether they in fact are tests for different behavior syndromes. Recently, it has been suggested that open field tests be used to measure exploration-avoidance behavior, while predator exposures be used to measure bold-shy behavior (Réale et al., 2007; Conrad et al., 2011; Carter et al., 2013). This study demonstrates that open field tests and predator exposures do not result in the same behavioral conclusion. While the open field tests resulted in significant differences in freezing probability between small and large fish, the predator exposures produced significant differences in freezing probability by diet type for small and large fish. Nevertheless, both tests have merit in assessing behavioral responses of hatchery-raised fish.

The study of hatchery fish behavior has growing interest as an important tool for assessing the effects of hatchery-rearing on the innate responses of fish (Conrad et al., 2011; Huntingford, 2004). With high prevalence and uniformity of the assessed behaviors across diet and size during the open field tests, Snake River cutthroat trout will likely show a wide variety of initial responses to their stocking environment, encompassing the spectrum of exploration-avoidance behavior. Snake River cutthroat trout do respond immediately to a predator attack, however it is unclear if these behaviors differ in intensity from that of wild cutthroat, and whether survival of predator encounters would differ between wild and hatchery cutthroat. Additional research will be necessary to determine these factors, and if diminished responses or survival are evident anti-predator training may be necessary for improvement. While there is evidence of hatchery salmonids habituating in both freezing and fleeing response to multiple avian predator attacks (Petersson and Jarvi, 2006), the current study shows evidence of habituation in only the fleeing response for small and large fish. Since predation risk only existed on one end of the tank, this could be an artifact of cutthroat remaining within a perceived safe distance from the predator strikes once they are out of striking distance. In regards to freezing response, the consistent response of the cutthroat in this study is promising. However, when implementing anti-predator training for hatchery fish using a model predator, stochasticity should be incorporated into the movement of the model in order to prevent desensitization.

While there is concern over artificial selection for faster growth impacting the behavior of hatchery reared salmonids (Huntingford, 2004), this study did not produce compelling evidence for similar concerns with Snake River cutthroat trout, at least for this particular strain. These fish are consistently wary of human interaction and exhibit caution both in new environments and in the presence of a novel predator. However, it is important to remember that cutthroat trout have not been as intensively cultured as other salmonids, and as such there has not been a strong selection for growth or habituation to human activity. These cutthroat exhibited lower growth rates and feed efficiencies than rainbow trout during the feed trial (See Chapter 2). While the apparent retention of caution and predator avoidance behavior would benefit cutthroat trout restoration efforts, engaging in selective breeding to improve growth performance must be approached cautiously, because such selection could negatively alter the behaviors of these fish.

6. Conclusions

In an open field environment, significant differences between small and large sub-adult Snake River cutthroat in freezing probability were observed. Snake River cutthroat trout exhibited anti-predator behaviors in response to simulated attacks by a model great blue heron, and significant differences in freezing response were observed between small and large fish fed different diet types. Specifically, higher likelihoods of freezing in fish fed BFTC Experimental or 45CP:16CL than fish fed Skretting Steelhead were observed, potentially due to differences in diet formulation. Evidence of a higher likelihood of darting in small trout than large trout and evidence of a diminished darting response in medium fish fed Skretting Classic Trout was also present. Additional research is necessary to determine if hatchery reared cutthroat trout differ in anti-predator response and survival than wild cutthroat trout.

Tables and Figures

Table 3.1. Probability of behaviors occurring by diet and size class (small or large) for sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during open field tests performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of groups within a column, unless otherwise stated. Probabilities with different superscript letters are significantly different (P < 0.05). Probabilities having the same superscript letters were not significantly different. The absence of letters indicates no significant difference between groups.

Diets	P(MP)[1]	P(FR)[2]	P(TH)[3]	P(SW)[4]	P(CE)[5]	P(RE)[6]	Exploration Rate**
40CP:12CL	0.93 ± 0.07	0.50 ± 0.13	1.00 ± 0.00	1.00 ± 0.00	0.86 ± 0.09	0.86 ± 0.09	0.23 ± 0.07
45CP:16CL	0.79 ± 0.11	0.50 ± 0.13	1.00 ± 0.00	1.00 ± 0.00	0.79 ± 0.11	0.71 ± 0.12	0.34 ± 0.09
45CP:24CL	0.86 ± 0.09	0.50 ± 0.13	0.93 ± 0.07	0.93 ± 0.07	0.71 ± 0.12	0.86 ± 0.09	0.27 ± 0.07
BFTC Experimental	0.86 ± 0.09	0.64 ± 0.13	1.00 ± 0.00	0.86 ± 0.09	0.50 ± 0.13	0.71 ± 0.12	0.20 ± 0.09
Skretting Classic Trout	0.86 ± 0.09	0.50 ± 0.13	1.00 ± 0.00	0.93 ± 0.07	0.71 ± 0.12	0.71 ± 0.12	0.32 ± 0.08
Skretting Steelhead	0.79 ± 0.11	0.57 ± 0.13	1.00 ± 0.00	0.86 ± 0.09	0.79 ± 0.11	0.50 ± 0.13	0.24 ± 0.07
Class							
Large	0.88 ± 0.05	0.67 ± 0.07[a]	1.00 ± 0.00	0.93 ± 0.04	0.71 ± 0.07	0.76 ± 0.07	0.28 ± 0.05
Small	0.81 ± 0.06	0.40 ± 0.08[b]	0.98 ± 0.02	0.93 ± 0.04	0.74 ± 0.07	0.69 ± 0.07	0.25 ± 0.04
Overall*	0.84 ± 0.04	0.54 ± 0.05	0.99 ± 0.01	0.93 ± 0.03	0.73 ± 0.05	0.73 ± 0.05	0.27 ± 0.03

* Not included in statistical analyses.
** Exploration rate (number of lines crossed/ total time) is shown as mean ± SEM.
[1] Probability of maintaining position.
[2] Probability of freezing.
[3] Probability of thigmotaxis.
[4] Probability of swimming.
[5] Probability of crossing center line of tank.
[6] Probability of returning to starting block.

Table 3.2. Probability of behaviors occurring by diet for medium sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during open field tests performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of groups within a column, unless otherwise stated. The absence of letters indicates no significant difference (P>0.05) between groups.

Diets	P(MP)[1]	P(FR)[2]	P(TH)[3]	P(SW)[4]	P(CE)[5]	P(RE)[6]	Exploration Rate**
40CP:12CL	0.71 ± 0.17	0.57 ± 0.19	1.00 ± 0.00	1.00 ± 0.00	0.71 ± 0.17	0.71 ± 0.17	0.38 ± 0.13
45CP:16CL	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.71 ± 0.17	0.86 ± 0.13	0.21 ± 0.09
45CP:24CL	1.00 ± 0.00	0.86 ± 0.13	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.09
BFTC Experimental	0.86 ± 0.13	0.43 ± 0.19	1.00 ± 0.00	0.71 ± 0.17	0.57 ± 0.19	0.57 ± 0.19	0.25 ± 0.13
Skretting Classic Trout	0.86 ± 0.13	0.57 ± 0.19	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.86 ± 0.13	0.24 ± 0.11
Skretting Steelhead	1.00 ± 0.00	0.57 ± 0.19	1.00 ± 0.00	1.00 ± 0.00	0.86 ± 0.13	0.86 ± 0.13	0.25 ± 0.09
Overall*	0.90 ± 0.05	0.67 ± 0.07	1.00 ± 0.00	0.95 ± 0.03	0.81 ± 0.06	0.81 ± 0.06	0.31 ± 0.04

* Not included in statistical analyses.
** Exploration rate (number of lines crossed/ total time) is shown as mean ± SEM.
[1] Probability of maintaining position.
[2] Probability of freezing.
[3] Probability of thigmotaxis.
[4] Probability of swimming.
[5] Probability of crossing center line of tank.
[6] Probability of returning to starting block.

Table 3.3. Probabilities of behaviors occurring for small and large sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during predator exposures performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of time periods within a column. Probabilities with different superscript letters are significantly different ($P < 0.05$). Probabilities having the same superscript letters were not significantly different. The absence of letters indicates no significant difference between time periods. Blank values were not measured during video analyses.

	P(Freeze)[1]	P(Dart)[2]	P(Thigmotaxis)[3]	P(MP)[4]	P(Swim)[5]
Pre-Exposure	0.10 ± 0.03[b]	0.00 ± 0.00[b]	1.00 ± 0.00	0.99 ± 0.01	0.57 ± 0.05
Exposure	0.70 ± 0.05[a]	0.52 ± 0.05[a]	0.99 ± 0.01	-	-
Post-Exposure	0.12 ± 0.04[b]	0.00 ± 0.00[b]	0.98 ± 0.02	0.98 ± 0.02	0.43 ± 0.05

[1] Probability of freezing.
[2] Probability of darting.
[3] Probability of thigmotaxis.
[4] Probability of maintaining position.
[5] Probability of swimming.

Table 3.4. Probabilities of behaviors occurring for medium sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during predator exposures performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of time periods within a column. Probabilities with different superscript letters are significantly different ($P < 0.05$). Probabilities having the same superscript letters were not significantly different. The absence of letters indicates no significant difference between time periods. Blank values were not measured during video analyses.

	P(Freeze)[1]	P(Dart)[2]	P(Thigmotaxis)[3]	P(MP)[4]	P(Swim)[5]
Pre-Exposure	0.05 ± 0.03[b]	0.00 ± 0.00[b]	1.00 ± 0.00	1.00 ± 0.00	0.52 ± 0.08
Exposure	0.90 ± 0.05[a]	0.36 ± 0.07[a]	1.00 ± 0.00	-	-
Post-Exposure	0.05 ± 0.03[b]	0.00 ± 0.00[b]	1.00 ± 0.00	1.00 ± 0.00	0.36 ± 0.07

[1] Probability of freezing.
[2] Probability of darting.
[3] Probability of thigmotaxis.
[4] Probability of maintaining position.
[5] Probability of swimming.

Table 3.5. Probability of behaviors occurring by diet and size class (small or large) for sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during predator exposures performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of groups within the first two response columns and mean ± SEM of groups within the last two response columns. Columns with superscript letters indicate statistical significance ($P < 0.05$) found in ANOVA. Probabilities or means having the same superscript letters were not significantly different in post-hoc testing. The absence of letters indicates no significant difference between groups.

Diets	P(Dart)[1]	P(Freeze)[2]	Average Total Dart Responses	Average Total Freeze Responses
40CP:12CL	0.71 ± 0.12	0.64 ± 0.13 [a]	1.50 ± 0.40	1.93 ± 0.49
45CP:16CL	0.50 ± 0.13	0.93 ± 0.07 [a]	0.86 ± 0.27	2.86 ± 0.43
45CP:24CL	0.50 ± 0.13	0.57 ± 0.13 [a]	1.07 ± 0.38	1.64 ± 0.45
BFTC Experimental	0.71 ± 0.12	0.93 ± 0.07 [a]	1.64 ± 0.40	2.29 ± 0.41
Skretting Classic Trout	0.29 ± 0.12	0.64 ± 0.13 [a]	0.57 ± 0.36	2.00 ± 0.51
Skretting Steelhead	0.43 ± 0.13	0.50 ± 0.13 [a]	0.79 ± 0.28	1.50 ± 0.49
Class				
Large	0.43 ± 0.08	0.76 ± 0.07	1.00 ± 0.23	2.33 ± 0.29
Small	0.62 ± 0.07	0.69 ± 0.07	1.14 ± 0.19	1.74 ± 0.25
Overall*	0.52 ± 0.05	0.70 ± 0.05	1.07 ±0.15	2.04 ± 0.19

*Not included in statistical analyses.
[1] Probability of darting.
[2] Probability of freezing.

Table 3.6. Probability of behaviors occurring by diet for medium sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* during predator exposures performed at the Bozeman Fish Technology Center in 2015. Values are probability ± SEM of groups within the first two response columns and mean ± SEM of groups within the last two response columns. The absence of letters indicates no significant difference (P>0.05) between groups.

Diets	P(Dart)[1]	P(Freeze)[2]	Average Total Dart Responses	Average Total Freeze Responses
40CP:12CL	0.57 ± 0.19	1.00 ± 0.00	1.00 ± 0.44	2.57 ± 0.53
45CP:16CL	0.43 ± 0.19	0.86 ± 0.13	0.86 ± 0.46	2.71 ± 0.71
45CP:24CL	0.43 ± 0.19	0.86 ± 0.13	0.43 ± 0.20	1.86 ± 0.70
BFTC Experimental	0.57 ± 0.19	0.86 ± 0.13	1.00 ± 0.44	3.00 ± 0.79
Skretting Classic Trout	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	3.86 ± 0.40
Skretting Steelhead	0.14 ± 0.13	0.86 ± 0.13	0.57 ± 0.57	2.00 ± 0.65
Overall*	0.36 ± 0.07	0.90 ± 0.05	0.64 ± 0.16	2.67 ± 0.27

* Not included in statistical analyses.
[1] Probability of darting.
[2] Probability of freezing.

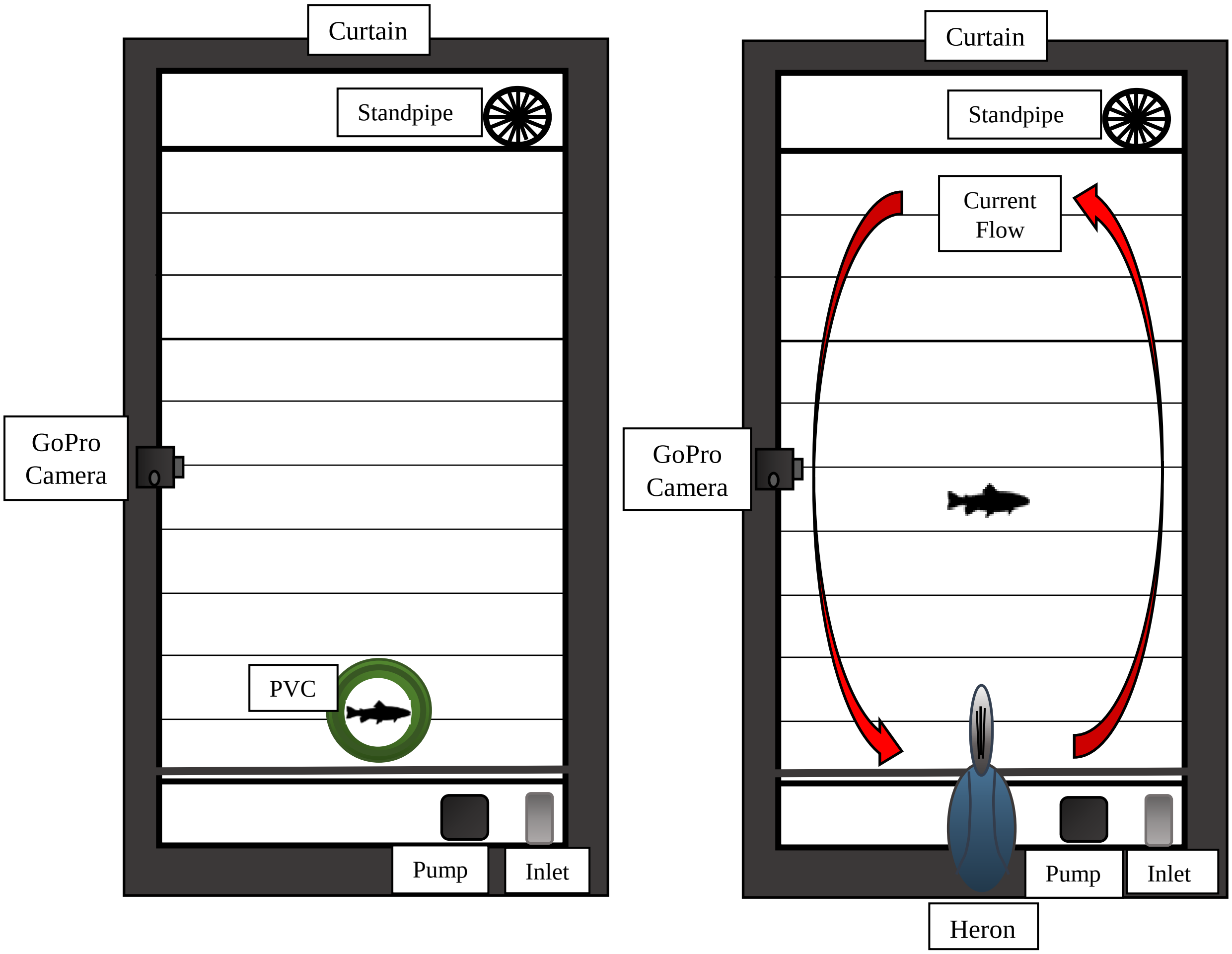

Figure 3.1. Schematic diagrams of open field test (left) and predator exposure trials (right) used in behavioral studies on sub-adult Snake River Cutthroat trout *Oncorhynchus clarkii behnkei* conducted at Bozeman Fish Technology Center in 2015.

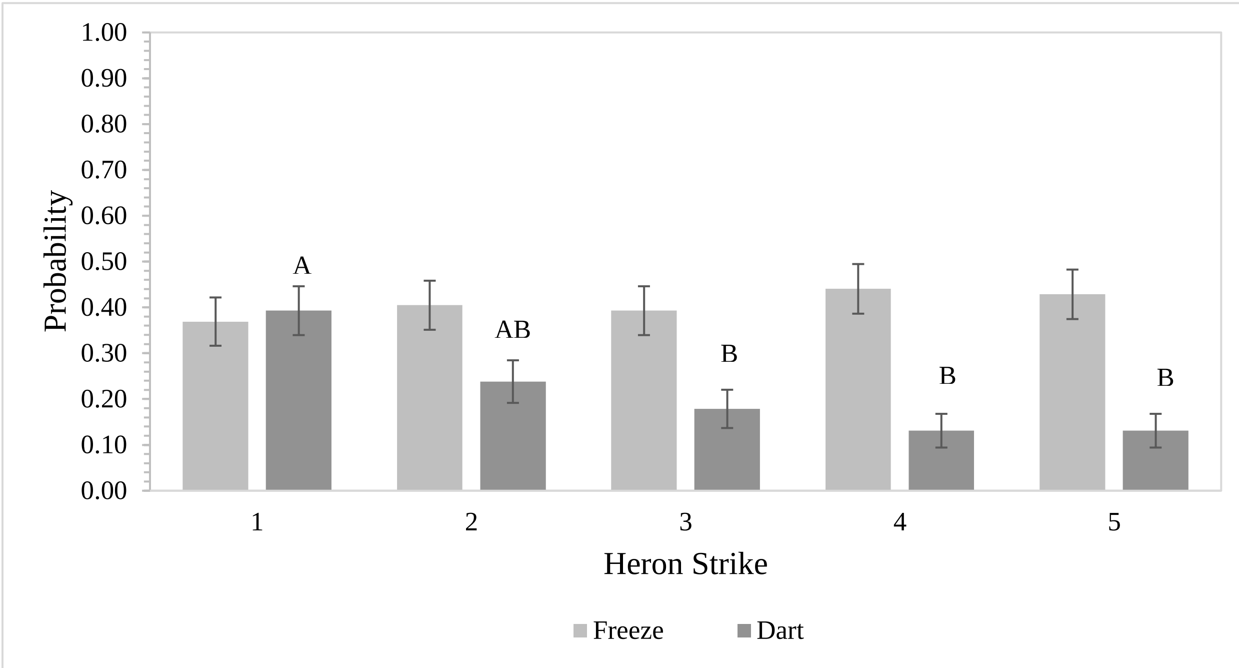

Figure 3.2. Probabilities ± SEM of freezing or darting behavior of small and large sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* occurring during multiple heron strikes. Probabilities with different letters are significantly different ($P < 0.05$). Probabilities having the same letters were not significantly different. The absence of letters indicates no significant difference between heron strikes.

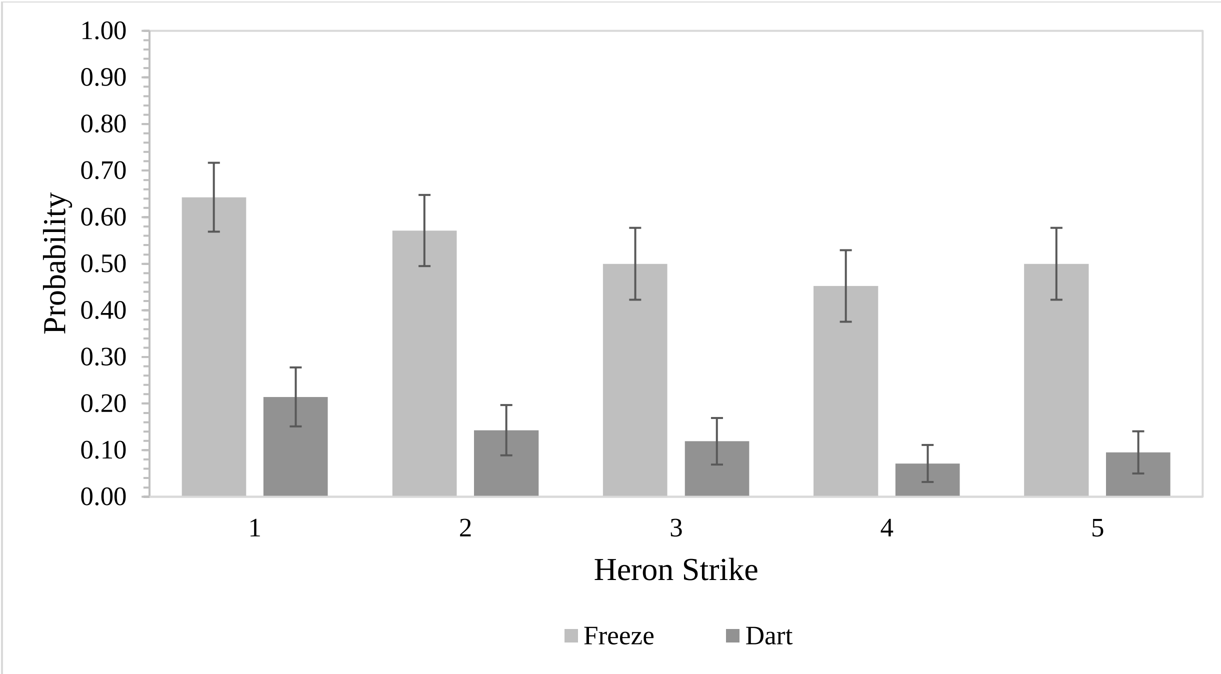

Figure 3.3. Probabilities ± SEM of freezing or darting behavior of medium sub-adult Snake River cutthroat trout *Oncorhynchus clarkii behnkei* occurring during multiple heron strikes. The absence of letters indicates no significant difference (P>0.05) between heron strikes.

REFERENCES

Alvarez, D., Nicieza, A.G., 2003. Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. Journal of Fish Biology 63, 1565–1577.

Alves, J., Krieger, D., Nesler, T., 2004. Conservation plan for Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) in Colorado. Colorado Division of Wildlife, Aquatic Wildlife Section, Denver, Colorado, USA.

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, 401– 411.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67 (1), 1– 48.

Benítez-Santana, T., Masuda, R., Juárez Carillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C.M., Izquierdo, M.S. 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparus aurata* larvae. Aquaculture 264 (1– 4), 408– 417.

Biro, P.A. Post, J.R., 2008. Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. Proceedings of the National Academy of Sciences of the United States of America 105(8), 2919– 2922.

Biro, P.A., Abrahams, M.V., Post, J.R., Parkinson, E.A., 2004. Predators select against high growth rates and risky behavior in domestic trout populations. Proceedings of the Royal Society of London Biology 271, 2233– 2237.

Brown, G.E., Ferrari, C.O., Malka, P.H., Oligny, M.A., Romano, M., Chivers, D.P., 2011. Growth rate and retention of learned predator cues by juvenile rainbow trout: faster growing fish forget sooner. Behavioral Ecology and Sociobiology 65, 1267– 1276.

Brown, C., Laland, K., 2001. Social learning and life skills training for hatchery reared fish. Journal of Fish Biology 59, 471– 473.

Brown, C., Laland, K., Krause, J., 2006. Fish cognition and behavior. Blackwell Publishing Ltd., Oxford, UK.

Brown, G.E., Smith, J.F., 1998. Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. Canadian Journal of Fisheries and Aquatic Sciences 55, 611– 617.

Brown, G.E., Smith, J.F., 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 75, 1916– 1922.

Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G., Heinsohn, R., 2012. Animal personality: what are behavioural ecologists measuring? Biological Reviews 88, 465– 475.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B., Sih, A., 2011. Behavioral syndromes in fishes: a review with implications for ecology and management. Journal of Fish Biology 78, 395– 435.

Ersbak, K., Haase, B.L., 2011. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3 (2), 142– 151.

Fox, J., Weisberg, S., 2011. An {R} companion to applied regression, second edition. Sage, Thousand Oaks, CA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gilmour, K.M., DiBattista, J.D., Thomas, J.B., 2005. Physiological Causes and Consequences of Social Status in Salmonid Fish. Integr. Comp. Biol., 45, 263– 273.

Glahn, J.F., Tomsa, T., Preusser, K.J., 1999. Impact of great blue heron predation at trout-rearing facilities in the Northeastern United States. North American Journal of Aquaculture 61, 349– 354.

Ham, B.R., Barrows, F.T., Huttinger, A., Duff, G.C., Yeoman, C.J., Maskill, M.G., Sealey, W.M., 2015. Evaluation of dietary soy sensitivity in Snake River cutthroat trout. North American Journal of Aquaculture 77 (2), 195– 2015.

Hozumi, N., Miyatake, T., 2005. Body-Size Dependent Difference in Death-Feigning Behavior of Adult *Callosobruchus chinensis*. Journal of Insect Behavior 18 (4), 557-566.

Huntingford, F.A., 2004. Implications of domestication and rearing conditions for the behavior of cultivated fishes. Journal of Fish Biology 65, 122– 142.

Ishizaki, Y., Masuda, R., Uematsu, K., Shimizu, K., Arimoto, M., Takeuchi, T., 2001.The effect of dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail. Journal of Fish Biology 58, 1691–1703.

JMP®, Version 11.2.1. SAS Institute Inc., Cary, NC, 1989– 2007.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software, 69 (1), 1– 33.

Lima, S.L., 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Advances in the Study of Behavior 27, 215–290.

Lima, S.L, Dill, L.M., 1989. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68, 619– 640.

Martín, J., López, P. 2003. Ontogenetic variation in antipredator behavior of Iberian rock lizards (*Lacerta monticola*): effects of body-size-dependent thermal-exchange rates and costs of refuge use. Canadian Journal of Zoology 81 (7), 1131-1137.

McNeil, W.J., 1991. Expansion of cultured Pacific salmon into marine ecosystems. Aquaculture 98, 173–183.

Miller, R.B., 1954. Comparative survival of wild and hatchery-reared cutthroat trout in a stream. Transactions of the American Fisheries Society 83 (1), 120– 130.

Mesquita, F., Young, R.J., 2007. The behavioral responses of Nile tilapia (*Oreochromis niloticus*) to anti-predator training. Applied Animal Behaviour Science 106, 144– 154.

National Research Council, 2011. Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Pettersson, L.B., Brönmark, C., 1993. Trading off safety against food – state-dependent habitat choice and foraging in Crucian carp. Oecologia 95, 353–357.

Petersson, E., Jarvi, T., 2006. Antipredator response in wild and sea-ranched brown trout and their crosses. Aquaculture 253, 218– 228.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biological Reviews, 82, 291– 318.

RStudio Team, 2015. RStudio: integrated development for R. RStudio, Inc., Boston, MA. http://www.rstudio.com/.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, The American Institute of Fishery Research Biologist, and the American Society of Ichthyologists and Herpetologists), 2004. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland, USA.

Vehanen, T., 2003. Adaptive flexibility in the behaviour of juvenile Atlantic salmon: short-term responses to food availability and threat from predation. Journal of Fish Biology 63, 1034–1045.

Wahle, R.A., 1992. Body-size dependent anti-predator mechanisms of the American lobster. Oikos 65, 52-60.