ASSESSMENT OF ALTERED REARING ENVIRONMENTS ON SURVIVAL AND PERFORMANCE OF HATCHERY-REARED TROUT: IMPLICATIONS FOR CUTTHROAT TROUT REINTRODUCTION PROGRAMS

by

Clinton James Smith

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

of

Master of Science

In

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2011

APPROVAL

of a thesis submitted by

Clinton James Smith

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to The Graduate School.

Dr. Thomas E. McMahon

Approved for the Department of Ecology

Dr. David W. Roberts

Approved for The Graduate School

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

Clinton James Smith

August, 2011

ACKNOWLEDGEMENTS

First off, I would just like to acknowledge what a wonderful opportunity and a blessing this project has been. An initial thanks goes to my family for their support, I love you all. There are many individuals who have given me much needed advice and assistance throughout the length of this project and I offer them my deepest gratitude. I would like to thank my advisor, Dr. Tom McMahon for selecting me for this research opportunity, hours of helpful review and edits, and for mentoring me throughout my time under his tutelage. I would like to thank Dr. Molly Webb for her service on my committee, her numerous ideas furthering my research, and for her understanding of the physiological aspects of this project. I would also like to thank Dr. Chris Guy for his service on my committee and his thorough understanding fisheries science. I would like to thank my funding source Montana, Fish, Wildlife and Parks. I would like to acknowledge the Bozeman Fish Technology Center, Sekokini Springs Natural Rearing Facility, and Crystal Lakes Fish Hatchery for their generous allowance of time and space, without which this project could not have proceeded. I would like to thank other individuals including Brian Marotz, Scott Relyea, Matt Boyer, Mark Kornick, Matt Toner, Jason Ilgen, and Eli Cureton for their assistance. I would like to thank all those who worked with me under the title of technician or independent researcher. Additionally, I would like to thank my fellow graduate students and MSU faculty for their assistance and support. To all those listed above, and those involved that I may have not mentioned, thank you.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF TABLES - CONTINUED

LIST OF TABLES - CONTINUED

LIST OF FIGURES

LIST OF FIGURES - CONTINUED

Figure Page

LIST OF FIGURES - CONTINUED

Figure Page

ABSTRACT

Reintroduction of native fish stocks is an important management tool used to mitigate the effects of invasive species and loss of habitat. A common reintroduction tool is the use of hatchery fish to create or supplement native populations in areas of their historic range. However, the low survival and poor performance of some hatchery outplants is an issue for hatchery reintroductions, as broodstocks may be limited. As a result, there has been a recent effort to develop 'conservation hatcheries' that employ hatchery rearing strategies that might improve the effectiveness of hatchery reintroduction efforts. This study developed and evaluated the effectiveness of enriched hatchery rearing strategies on increasing effectiveness of hatchery reintroductions for inland salmonids. Three hatchery rearing treatments of varying complexity were developed for rearing trials using westslope cutthroat trout *Oncorhynchus clarkii lewisi* and rainbow trout *Oncorhynchus mykiss*. Fish were reared in the hatchery for 60 days and various performance assessments were compared to evaluate behavioral, morphological, and physiological differences among the rearing treatments. A second 60 day rearing period using westslope cutthroat trout was followed by an outplant assessment. Fish were reared in the same three hatchery treatments and at the end of the hatchery-rearing period fish were placed into rearing ponds. Outplanted fish were left in the ponds with no artificial feed or predation relief. After 2 months in the ponds, fish were removed and their survival and growth were analyzed. Growth and survival in the hatchery were similar among the rearing treatments for both species. The most complex treatment was associated with increased cover seeking behavior, reduced aggression, higher fin condition, and improved coloration. Survival results from the outplanted fish were compromised because of fish movement; however, there was evidence that the most complex treatment may have performed better as suggested by growth data and proximate analysis of lipid content. Our results suggest that alterations to the hatchery environment may improve the effectiveness of native species reintroduction efforts using hatchery-reared fish. Further research is needed to assess additional hatchery-rearing environment alterations as well as the long-term effects such alterations might have on hatchery outplanted fish.

INTRODUCTION

As native salmonids continue to be threatened by nonnative species and loss of habitat, reintroduction of native populations into historic habitat is imperative to recovery efforts. Currently, there are two main reintroduction approaches used for the recovery of native species. Outplanting of hatchery-reared juveniles has historically been the most common method of population recovery; however, numerous findings have identified several adverse effects caused by hatchery rearing that may outweigh the benefits (e.g., Hilborn 1992; White et al. 1995; Weber and Fausch 2003). The present trend for reintroduction is the use of remote site incubators (RSIs). Remote site incubators are placed onsite, where fertilized eggs incubate and the emergent fry volitionally colonize streams or lakes (Kaeding and Boltz 2004). RSIs are advantageous as wild fry are produced onsite, fry emergence rates are high, fry can imprint to natal waters, and they are cost-effective. RSI use in the Pacific Northwest is prevalent as part of the effort to rehabilitate salmon and trout populations (Manuel et al. 1991, cited in Kaeding and Boltz 2004), however there have been few in-depth evaluations of their success as a population recovery tool (Kaeding and Boltz 2004). Potential problems with RSIs include sedimentation, fungal infection, and egg fertilization success (Kaeding and Boltz 2004). Also, the typical high mortality of emergent fry in the wild may limit the number of spawning adults produced via this method.

The primary benefit to hatchery reintroduction of native species is efficiency; under the controlled conditions of a hatchery, the same number of parents can produce considerably more offspring than they could in the wild (Ford and Hard 2000). The potential downside of using hatchery-reared juveniles includes poor survival and competition with wild stocks, as discussed by White et al. (1995) and Einum and Fleming (2001). Although competition with wild stocks is not a concern for some reintroduction efforts, the differences between hatchery and wild fish are, as it is thought that these differences may account for poor survival seen among outplanted juveniles.

Traditionally, fish culture has focused on the development of rearing practices that support health and growth in the hatchery and thus increase production (White et al. 1995). The sometimes poor survival of outplanted fish has generally not been a limiting factor because of high production and abundant broodstocks. However, overcoming the low survival of outplanted fish for population recovery purposes poses more of a challenge, as broodstocks are rare and production capacity is usually limited. Also, producing more fish does not address the behavioral, morphological, physiological, and genetic differences that are likely the causes for the sometimes low survival of outplanted juveniles. Brown and Day (2002) found that most mortality of outplanted juveniles occurs within the first 10 days after release, suggesting that adaptation to different environments is crucial. The increasing evidence of negative interactions between hatchery-reared and wild fish (Weber and Fausch 2003) and the potential harmful effects of hatchery-reared fish (White et al. 1995) have led to a re-evaluation of hatchery practices (Mobrand et al. 2005). The re-evaluation of hatchery practices seems to coincide with an expansion of hatcheries' purpose from support of recreational and commercial fisheries to support of native species recovery programs. The shift to native species recovery programs has caused research to focus on the development and evaluation of hatchery practices that attempt to produce fish that closely mimic traits of wild fish and thereby minimize the potential harmful effects associated with hatchery rearing and that improve survival of outplanted juveniles (Maynard et al. 1995; Brown and Laland 2001; Brown and Day 2002).

Hatchery-reared fish may differ genetically, behaviorally, morphologically, and physiologically from wild fish (Allendorf and Phelps 1980; Hindar et al. 1991; White et al. 1995; Weber and Fausch 2003). The differences between hatchery fish and wild fish have been termed ‘deficits,’ assuming that producing a fish with similar traits and fitness to a wild fish is the ultimate goal. Genetic divergence between wild and hatchery stocks arise from artificial selection that occurs throughout the hatchery process (Hindar et al. 1991; Einum and Fleming 1997; Gross 1998). Artificial selection causes the expression of traits that may be selected against in the wild (Weber and Fausch 2003), thus making hatchery fish less fit when outplanted. Genetic divergence can be reduced by the use of local-origin broodstocks, continued introduction of wild fish genetics in broodstocks, and increased phenotypic similarity between hatchery and wild fish (Kostow 2009). For example, McMullin and Dotson (1988) demonstrated that by adopting relatively simple procedures, a fully wild gene pool can be maintained among hatchery broodstock.

The effect of behavioral deficits on fitness and survival are much less certain than those of genetic deficits. Behaviorally, hatchery-reared salmonids tend to be more aggressive (Fenderson et al. 1968; Mesa 1991; Berejikian et al. 1996, 1999), have poorer predator avoidance (Berejikian 1995; Johnsson et al. 2001), and have inappropriate habitat selection and foraging behavior (Bachman 1984; Mesa 1991; Deverill et al. 1999) when compared to wild fish, traits which many fisheries scientists presumably link to the poorer post-stocking survival seen among outplanted salmonids (Wills 2006). These behavioral deficits may be short lived, however, as evidenced by the high survival rates of hatchery fish in some waters (Wiley et al. 1993a). Other studies have shown that hatchery fish lose weight and cease feeding after stocking (Miller 1954; Usher et al. 1991), but then resume normal feeding and growth within a few weeks after release as they become acclimated to the new environment and learn to identify new prey (Johnsen and Ugehahl 1989). Also, a brief exposure to predator stimuli can improve predator avoidance by hatchery fish in the wild (Berejikian et al. 1995, 1999; Olla et al. 1998; Vilhunen 2006). These findings suggest that a learning component is important to the development of behaviors necessary for survival in the wild.

The realization that learning and imprinting are important to the development of proper behaviors in hatchery-reared fish is a recent trend. In other captive breeding programs, such as those with endangered birds and mammals, pre-release training has been used to maximize survival after release (Brown and Laland 2001; Brown and Day 2002). Hatchery fish have been thought to have innate behaviors brought about by the artificial selection for behaviors to optimize hatchery survival. Learning and imprinting of hatchery fish has not generally been considered. Several studies have shown that behavioral differences occur between hatchery fish and wild fish within a few days after emergence (e.g., Vincent 1960; Moyle 1969), suggesting that such behaviors are innate. However, recent studies have shown that predator avoidance, feeding, and social behavior may have a larger learning component than previously thought and demonstrate the importance of early learning on behavior (Olla et al. 1998; Brown and Laland 2001; Brown and Day 2002; Lee and Berejikian 2008). Many behaviors require some degree of learning which can only come about via repeated exposure to stimuli (Brown and Day 2002). For example, simple differences in food delivery and cover can produce drastically different behaviors among juvenile rainbow trout *Oncorhynchus mykiss*, with more natural conditions producing hatchery fish that more closely mimic traits of wild fish (Berejikian et al. 2000; Rick Barrows, U.S. Department of Agriculture, personal communication). This evidence suggests that hatchery fish can be trained to behave more like wild fish, and as a result, possibly have increased fitness in a natural environment.

Morphological and physiological deficits also likely play a role in the success of outplanted hatchery fish. Hatchery-reared fish may differ morphologically from wild fish by having poor fin condition (Latremouille 2003; Berejikian and Tezak 2005) and unnatural coloration (Maynard et al. 1995; Berejikian et al. 1999, 2001). Fin condition has been identified as a key indicator of overall health and post-stocking survival of salmonids (Wagner et al. 1997; Latremouille 2003). Abnormal coloration increases predation risk and reduces camouflage and reproductive success (Berejikian et al. 1999, 2001). Coloration also plays a role in the social interactions of salmonids as position in the social hierarchy is signaled by coloration, with the paler colors typical of hatchery fish signaling dominance (Newman 1956; Berejikian et al. 1999). Physiologically, hatchery fish tend to have decreased swimming ability (Vincent 1960; McDonald et al. 1998), reduced cortisol response to a stressor (Woodward and Strange 1987), and lower metabolic responses to heat stress when compared to wild fish (Werner et al. 2006).

Anderson (1990) found that the immune function in fish can be compromised by stress, which suggests that the stressors from both hatchery-rearing and outplanting may inhibit disease resistance. The stress from transportation and direct release into new environments may also contribute to the low survival of outplanted fish (Olla et al. 1998).

As conservation propagation programs increasingly become important tools in native species recovery, minimizing the aforementioned deficits and consequently increasing post-stocking survival is key. To date, many strategies to address these issues have been tested including rearing at lower densities, using more natural substrate, adding cover, utilizing more natural food delivery systems, improving food quality, anti-predator conditioning, swimming exercise, and 'soft release' practices (Wiley et al. 1993b; Maynard et al. 1995; Berejikian et al. 1999; Brown and Day 2002). 'Soft release' practices involve rearing fish in a hatchery setting and then acclimating the fish to a semi-natural environment (e.g., pond or natural stream section) for a short time period prior to outplanting. In the Pacific Northwest, a Natural Rearing and Enhancement System (NATURES) has been initiated in order to develop and evaluate new fish culture technologies to produce Pacific salmon *Oncorhynchus spp.* which are behaviorally, physiologically, and morphologically similar to their wild counterparts (Maynard et al. 2003). These methods are being adopted by conservation hatcheries throughout the Pacific Northwest, where, as of 2003, there were 27 anadromous salmon hatcheries implementing conservation hatchery programs (Anonymous 2003).

Findings from numerous NATURES studies (Maynard et al. 2003) suggest that the deficits of hatchery-reared salmonids can be reduced by using more complex, ‘enriched’ rearing environments. For example, hatchery fish reared in enriched environments consisting of gravel substrates, in-stream structure, and overhead cover had up to 50% higher survival than fish reared in conventional raceways with no complexity (Maynard et al. 2003). Studies using enriched environments have shown improved competitive ability, more natural growth rates, more natural exploratory behavior, and less variation in behavior (Berejikian et al. 2000, 2001; Lee and Berejikian 2008). Enriched environments may also result in improvement in morphological traits such as coloration and fin condition (Donnelly and Whoriskey 1991; Maynard et al. 1995, 2003; Berejikian and Tezak 2005). Brockmark et al. (2010) found that reducing rearing density to natural levels produced fish that were better at finding prey, more likely to consume novel prey, better at avoiding predators, and more likely to survive than fish reared at traditional hatchery densities. Stress levels have been shown to be reduced when overhead cover is present, which may therefore improve outplanting survival and disease resistance (Pickering et al. 1987). Also, prior experience of hatchery fish in a rearing environment that closely mimics a natural environment might lead to a reduced stress response when outplanted (Schreck 2000) compared to a fish without prior (i.e., those raised in a conventional hatchery rearing environment).

Not all conservation hatchery techniques have shown improvements in the performance of hatchery-reared fish. For example, Hawkins et al. (2007) found that predator training of Atlantic salmon *Salmo salar* smolts produced no difference in survival compared to untrained smolts. Other studies have shown that differences in hatchery-rearing environment had no effect on the rates of aggression and feeding of hatchery-reared steelhead *Oncorhynchus mykiss* fry (Riley et al. 2005). Also, hatchery steelhead fry reared in enriched environments consisting of submerged and overhead cover, underwater feed delivery, and cobble substrate may have negative effects on wild steelhead fry by increasing the aggressive behavior and decreasing the foraging rate of the wild fry (Tatara et al. 2008). Studies measuring survival of outplanted juveniles reared under enriched conditions are few and have also produced mixed results. Hatchery steelhead fry from both conventional and enriched rearing environments grew as well as naturally-reared fry but exhibited higher mortality (Tatara et al. 2009). Tatara et al. (2009) also found that steelhead fry reared in enriched hatchery environments with overhead and submerged cover and an underwater feed delivery system did not exhibit improved growth or survival after release compared to fish from conventional hatchery environments. Experiments examining the effectiveness of soft release practices have produced no clear trends to date. Conventionally-reared brown trout *Salmo trutta* exposed to a short pond rearing period with natural foods exhibited higher survival rates than those stocked directly into receiving waters (Hesthagen and Johnsen 1989). In contrast, Kostow (2004) found that steelhead reared in a hatchery had decreased survival after being reared in a semi-natural pond prior to release.

I reviewed a selection of literature pertaining to the effects of enriched hatchery rearing strategies on salmonids from the years 1989 to 2010 (Table 1). Of the studies summarized, only four of the 16 include non-anadromous salmonids and, of those four, only one concerned a North American species. Most studies focused on behavioral differences (11 studies), followed by survival (7, both in hatchery and post-stocking), growth (3), and morphological measures (1). Of the behavioral studies, eight examined social and/or agonistic behaviors and three examined predator avoidance. While the results from the studies are mixed, there are some noticeable trends. For example, social behavior (i.e., competitive ability, aggression, exploratory behavior) can be altered through hatchery rearing to more closely mimic the behaviors of wild fish, and hatchery fish can be trained to better avoid predators. There were no clear effects on growth or survival in the hatchery. The lack of post-stocking evaluation is also apparent among the literature as only seven mention any post-release assessments. Of the studies that performed post-release assessments, six compared survival, four of which found no survival improvements among fish from enriched rearing environments. This literature review demonstrates the need for enriched hatchery studies on inland salmonid species of concern and the lack of information on the lifelong survival and performance implications of enriched hatchery rearing strategies.

Table 1: Summary of literature reviewed examining various enriched hatchery rearing strategies and their effects on salmonid species. The term conventional refers to barren hatchery environments, semi-natural and enriched refer to hatchery environments with structure and cover, and naturally-reared and wild fish refer to raising fish in controlled stream sections and/or captured fish from the wild.

Reference	Species	Study Objectives	Findings
Hesthagen and Johnsen (1989)	Brown trout *Salmo trutta*	Compared lake survival of stocked hatchery fish and pre-stocked pond fish.	Significantly more pre-stocked pond fish recaptured than hatchery fish after 2 years.
Donnelly and Whoriskey (1991)	Brook trout *Salvelinus fontinalis*	Examined levels of avian predation on fish reared in tanks with different coloration.	Predation levels were lower among the fish reared in tanks with coloration similar to the predator arena.
Berejikian et al. (1999)	Chinook salmon *Oncorhynchus tshawytscha*	Examined the effects of exposure to chemical predator stimuli on juvenile hatchery fish.	Fish exposed to chemical stimuli learned predator recognition and thus spent more time motionless when exposed to a predator.
Berejikian et al. (2000)	Steelhead *Oncorhynchus mykiss*	Compared social dominance, growth, and habitat used between enriched and conventionally reared hatchery fish.	Fish reared in enriched environment were socially dominant and grew better in a quasi-natural environment, no difference in habitat use.
Berejikian et al. (2001)	Steelhead	Compared competitive ability of conventional, enriched, and naturally-reared fish.	Enriched and hatchery-reared fish had similar competitive ability; naturally-reared fish had greater territory overlap than the hatchery-reared fish.
Kostow (2004)	Steelhead	Exposed hatchery fish to semi-natural pond prior to outplanting and compared survival between hatchery and naturally-reared fish.	Rearing in a semi-natural pond was associated with decreased smolt-adult survival compared to naturally-reared fry.

Table 1 Continued

Berejikian and Tezak (2005)	Steelhead	Compared the dorsal fin height of fish from a conventional and enriched hatchery rearing environment and from a natural stream.	Fish from the enriched hatchery rearing environment and the natural stream had significantly greater fin height than did fish from the conventional hatchery environment.
Riley et al. (2005)	Steelhead	Compared aggression and feeding of naturally-reared and hatchery-reared fish from conventional and enriched environments at different densities.	Attack rate increased with density. Aggression and feeding rate were not affected by rearing environment.
Hawkins et al. (2007)	Atlantic salmon *Salmo salar*	Examined the influence of anti-predator conditioning on survival in the wild.	No difference in survival was found between hatchery and treatment fish exposed to anti-predator conditioning.
Fast et al. (2008)	Chinook salmon	Compared the post-release survival of fish from conventional and enriched rearing environments.	Found no evidence of differences in survival between the rearing environments.
Lee and Berejikian (2008)	Steelhead	Examined the behavior of fish from barren, structured, and structurally variable environments.	Structured environments were associated with increased exploratory behavior. Less behavioral variation was found in structured environments than in the barren environments.

Table 1 Continued

Tatara et al. (2008)	Steelhead	Compared aggression, feeding, and territoriality from wild, enriched, and conventionally reared fish.	No differences in aggression, foraging, or territoriality were found among treatments. Enriched fish mixed with wild fish was associated with lower foraging and aggression in wild fish.
Tatara et al. (2009)	Steelhead	Compared growth, survival, and habitat use of enriched and conventionally reared and examined the effects on wild fish.	No difference was found in growth in a stream among the wild, enriched, or conventional fish. No improvement in growth or survival was seen in the enriched fish. Enriched and conventionally reared fish have similar effects on wild fish.
Turek et al. (2009)	European grayling *Thymallus thymallus*	Examined growth and dispersal of hatchery-reared and pond reared fish and compared with wild fish.	Recapture rates and site fidelity were highest among wild fish. No difference in overall performance was found between the stocked fish.
Brockmark et al. (2010)	Brown trout	Examined behavior and survival among fish reared at three different densities in varying levels of tank structure.	Fish reared at natural densities were more likely to find prey in a maze, more likely to consume novel prey, and showed higher predator avoidance behavior. Fish reared at lower densities were two times more likely to survive than fish reared at high densities. Structure was found to have no clear treatment effects.

Table 1 Continued

Brockmark and Johnsen (2010)	Brown trout	Examined how rearing density influences social dominance, post-release growth, and survival.	Fish reared at natural densities had higher dominance, grew faster, and survived better than fish reared at higher densities.

Although initial results of enriched hatchery environments are encouraging, evaluation of which conservation hatchery strategies improve post-stocking survival of hatchery fish and enhance effectiveness of recovery programs is still in its infancy. Key aspects of this evaluation should include 1) testing of the fundamental assumption that improved rearing techniques will decrease environmentally induced behavioral, physiological, and morphological deficits expressed by hatchery fish and 2) that reducing these deficits through the use of enriched hatchery environments substantially improves survival in the wild (Berejikian et al. 2000; Brown and Day 2002).

A major recovery goal in Montana is to reintroduce westslope cutthroat trout *Oncorhynchus clarkii lewisi* into its historic habitats (MFWP 2007). It is estimated that westslope cutthroat trout currently occupy 19% (Van Eimeren 1996) to 39% (Shepard et al. 2003) of their historic range in Montana and that genetically pure westslope cutthroat trout currently occupy only 2.5% (Liknes and Graham 1988) to 9% (Shepard et al. 2003) of their historic range. Montana Fish, Wildlife and Parks (MFWP) has initiated an extensive reintroduction program named the South Fork Westslope Cutthroat Project (MFWP 2006). The project plans to chemically remove nonnative Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* and rainbow trout from 21 lakes in the South Fork Flathead River drainage for the purpose of preventing hybridization with the genetically pure westslope cutthroat trout that are native to the drainage. As part of the project, MFWP has purchased a former private hatchery, Sekokini Springs, and plans to develop a westslope cutthroat trout conservation hatchery for the purpose of producing drainage-specific broodstocks for reintroduction. However, there has been a lack of research evaluating the effectiveness and post-stocking survival of potamodromous salmonids, as most conservation hatchery work has focused on anadromous salmonids in the Pacific Northwest. The purpose of this study was to develop and evaluate various hatchery strategies for improving post-release survival of westslope cutthroat trout in order to maximize effectiveness of reintroduction efforts. I tested the hypotheses that the use of more complex hatchery rearing environments will improve morphology, behavior, and physiology of westslope cutthroat trout and enhance survival in the wild. The unique set of data pertaining to the effects of enriched hatchery rearing environments collected from rainbow and westslope cutthroat trout allowed me to also test the hypothesis that different species of varying levels of domestication would respond similarly to enriched hatchery rearing strategies. Findings from this study will be used to help develop what is believed to be the first conservation hatchery specifically designed for reintroducing a threatened, non-anadromous salmonid.

METHODS

Effects of Rearing Environment on Hatchery Performance

Study Populations

An initial assessment of rearing treatments and measures of hatchery fish performance were conducted with Arlee strain rainbow trout from Ennis National Fish Hatchery in spring 2009. The Arlee rainbow trout hatchery broodstock was first developed in 1978 and has continued to be artificially propagated with no new genetic infusion (Sean Henderson, Ennis National Fish Hatchery, personal communication). After developing methods using rainbow trout, the first rearing test used embryos of a wild strain westslope cutthroat trout broodstock obtained from Washoe Park Trout Hatchery, Anaconda, Montana. The MO12 westslope cutthroat trout stock at Washoe Park was first developed from wild fish in 1984 (Grisak and Marotz 2003). This stock first received genetic infusion from wild fish in 2003 and had experienced multiple infusions between 2003 and the time of this study (MFWP 2004; Scott Relyea, Montana Fish, Wildlife, and Parks, personal communication). A total of 15,000 MO12 eyed embryos were incubated in heath trays beginning 27 May 2009 until swim up was observed, at which point fish were randomly assigned to the treatment tanks and the remaining fry were placed into stock tanks.

A second test examined a domesticated strain of hatchery rainbow trout (Arlee strain) obtained as embryos from Ennis National Fish Hatchery. Performing the hatchery environment tests on two species of trout of different domestication levels provided a unique opportunity to compare the effects of hatchery rearing environment on different species and differing levels of domestication. Approximately 15,000 rainbow trout embryos were incubated in Heath trays beginning 12 December 2009 until swim up was observed, at which time fry were randomly assigned to each treatment tank and the remaining fry placed into holding tanks for later use as live prey for predator training.

Rearing Treatments

Three rearing environments varying in cover availability and food delivery system were tested at the Bozeman Fish Technology Center (BFTC). Water temperature was the same in all tanks and varied from 12 to 15 °C throughout the rearing trials. Water flow through the tanks was standardized at 7.5 L/min. Cleaning occurred daily and consisted of draining the tanks while using a broom to sweep out the debris in each tank. All tanks used for the rearing environments were 88 L (122 x 35 x 20 cm) aluminum rectangular troughs and covered with screens made of white plastic with 0.5 mm mesh to prevent emigration from the rearing tanks. Water depth was 17 cm as maintained by a stand pipe. Rearing treatments of increasing complexity were conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) (Figure 1). The conventional rearing treatment simulated conventional hatchery rearing wherein fish were hand fed and there was no overhead or submerged cover provided. The INT rearing treatment had overhead cover over outflow half of the tank and food was delivered via surface belt feeders programmed to 12 hour cycles. The overhead cover was made of black plastic sheeting placed over the outflow half of the tank. In addition to overhead cover, the ENR treatment included submerged cover and an underwater food delivery system. The

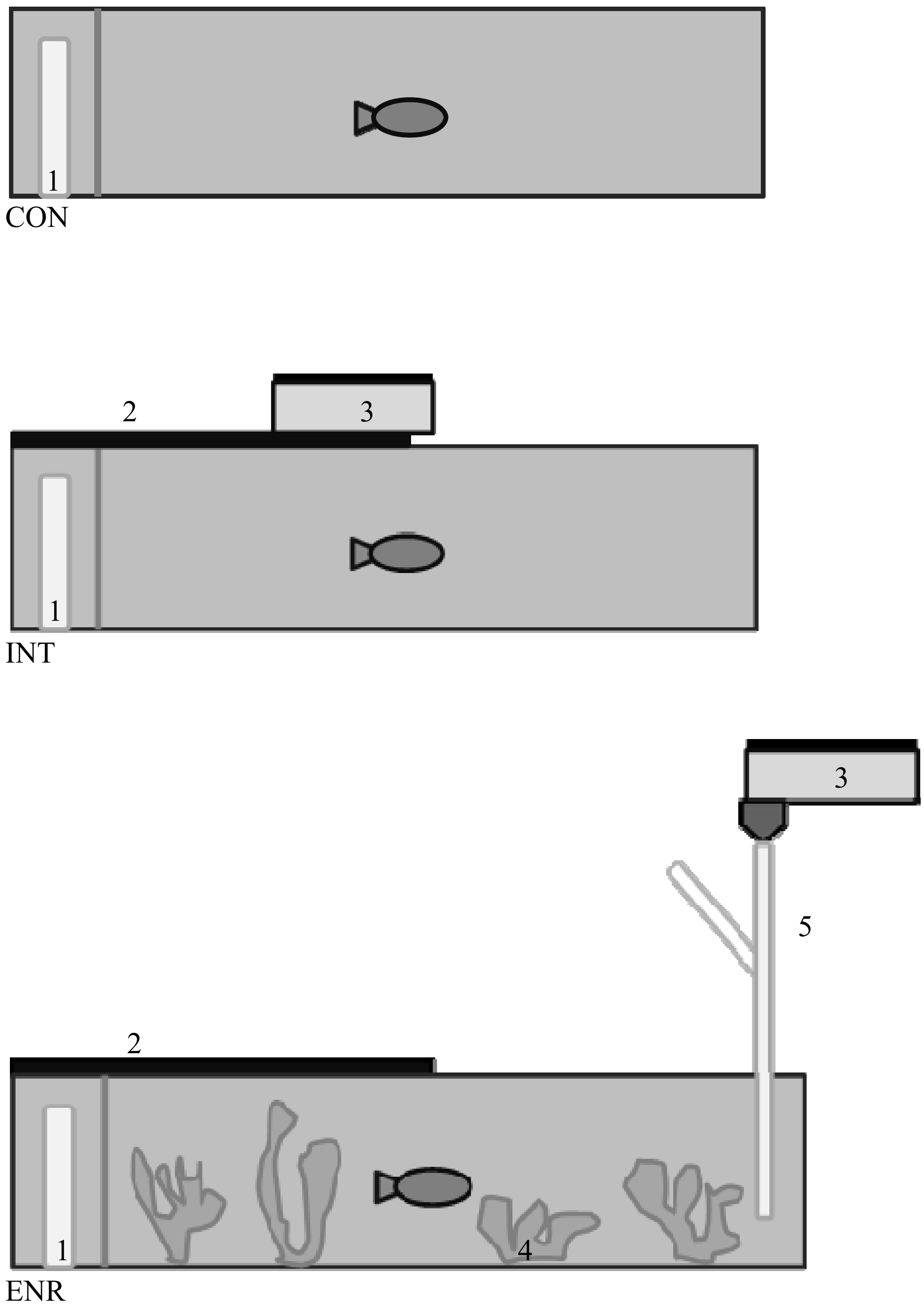


Figure 1: Diagram of three hatchery rearing treatments. Conventional (CON) rearing treatment without cover and fed by hand with water flow through a standpipe (1); the enriched-intermediate treatment (INT) with overhead cover (2) and a surface belt feeder (3); and the enriched-high treatment (ENR) with overhead and submerged cover (4) and an underwater feed delivery system consisting of a belt feeder and water inlet tube (5).

submerged cover was comprised of 8 separate cover units, each constructed from 20 x 7.5 cm strips of AquaMat®, bundled into groups of three strips attached to weights so as to remain submerged, but still movable during daily tank cleaning. The underwater food delivery system consisted of an elevated 12 hour belt feeder delivering food into a funnel system with an inflow pipe and submerged outlet which dispersed food pellets throughout the tank. There were four replicates of each rearing treatment for a total of 12 tanks. Initial fish density was 350 fish per tank (approximately 4 fish/L). Fish were fed to satiation as determined from approximately 3% body weight per day (Jason Ilgen, U.S. Fish and Wildlife Service, personal communication). Food ration was based on bulk weight measurements and the number of fish per tank as determined at day 0 and day 30 of the trial. The food ration for the CON treatment was delivered via five separate hand feedings on weekdays and two on the weekends, a typical hatchery feeding schedule. The feed for the INT and ENR treatments was placed onto 12 hour belt feeders each weekday morning and approximately 8 hour belt feeders on the weekends. Fish were fed BioVita starter pellets (Bio Oregon ®) in all rearing trials. Fish were reared in the treatment tanks for 60 days.

Survival and Growth

Survival was monitored throughout the 60-day rearing period with the number of mortalities recorded each day. Any mortalities occurring within the first week after study initiation were replaced in order to account for mortality associated with initial handling of young fry. Growth was analyzed by measuring the average length (mm) and weight (g) of individual fish from each rearing tank. Individual length and weight measurements were recorded for 30 fish per tank at 30 days and 60 days. The average initial length was 26 mm for cutthroat trout and 25 mm for rainbow trout. Both species initial weight was approximately 0.2 g. Fish condition was measured using Fulton's condition factor (K) according to Anderson and Neumann (1996) as follows:

$$K = (W / L^3) \times 100{,}000$$

where K is the condition factor, W is the weight (g), and L is the length (mm).

Dorsal Fin Index

Fin erosion is common among hatchery fish (Bosakowski and Wagner 1994) and has been linked to their overall health (Latremouille 2003). The dorsal fin height was compared among treatments using a modified Kindschi fin index (Kindschi 1987), an index of the proportion of dorsal fin height to total fish length. I used fork length instead of total length because of possible erosion of the caudal fin tips among test fish. At the end of the 60-day rearing trials, 30 fish were randomly selected from each tank, anesthetized with MS-222, and the dorsal fin height was measured.

Coloration

Hatchery fish may have different coloration than wild fish (see Maynard et al. 1995). To test for coloration differences among rearing treatments, I photographed 10 randomly selected fish from each tank with a Nikon digital camera and a 60 mm close-up lens. Coloration and spotting pattern for each fish was ranked using a 5-point scale (Table 2).

Behavior

Behavioral differences among the rearing treatments were examined with several different behavioral assessments. First, a general fright response of fish to a human observer was noted during daily cleanings (approach versus avoidance). Behavioral performance was also evaluated by examining cover use, territoriality, and predator avoidance. Each of these behavioral trials is described in detail below.

Cover: Cover use was compared among rearing treatments by placing fish in a 2.45 m-long, 61 cm-wide trough with overhead cover at one end. There was no water flow and the depth was set at 40 cm. The overhead cover was a 50 cm-long piece of Styrofoam placed near the water surface. The test tank was divided into 30 cm grid line sections so that the relative position of the fish could be recorded. Observations of fish distribution were made from behind a black plastic shroud. Two overhead lights provided uniform lighting within the test tank. Ten fish were introduced into the end of the tank opposite of the cover and their positions in the tank were recorded every 30

Table 2: Qualitative assessment scores used to determine fish coloration of rainbow trout and westslope cutthroat trout reared under different hatchery environments.

Score	Description
1	Lack of depth in color, light-silvery color, no coloration along lateral line, poor definition of parr marks and spotting
2	Little variation in color, light green body, no/little coloration along lateral line, parr marks scarcely visible, light spotting present
3	Drab green color, little coloration along lateral line, parr marks and spotting visible, but do not stand out
4	Color is green & bright, red hue along lateral line, parr marks and spotting evident
5	Deep, vibrant, dark green color, red coloration along lateral line, clear parr marks and spotting

seconds for three minutes using a voice recorder. The fish used in each trial were randomly selected from a single rearing tank for each trial. At the end of each trial, the fish were removed and the experimental tank was drained, cleaned, and refilled. Eight trials were performed for each of the three treatments. The proportion of fish using cover and the distribution of fish within the tank was compared among the treatments.

Territoriality: Aggression and territoriality were analyzed by placing six similarly-sized fish (70 ± 3 mm) in a 105 L-tank (30 x 46 x 76 cm) with a simulated riffle-pool design. For each trial, the six fish were randomly drawn from a single rearing tank. There were 2 trials for each treatment tank for a total of 24 trials. The tank was constructed of wood, sealed with a nontoxic epoxy, and a glass observation window fitted on one side. Water flow was maintained at 15 L/min. The test tank was surrounded by a black plastic shroud to shield the observer and prevent disturbance of test fish. Trials took place over two days, though the measurement period was only 24 hours. A submerged food delivery similar to the one used in the ENR rearing treatment tank delivered 1.5 g of feed each day to the test fish. The number of agonistic acts (nips, charges, chases, and lateral displays ([Table 3]; Keenleyside and Yamamoto 1962) were counted during three 10-minute observation periods: at 1, 22, and 24 hours after trial initiation. The first observation period was performed in an attempt to document aggression levels during the establishment of the social hierarchy. The observation periods at 22 and 24 hours were performed to assess aggression after full acclimation to the test tank. Dominance was determined by tank position, coloration, and social behavior.

Table 3: Description of behaviors assessed during agonistic behavior experiments using rainbow trout and westslope cutthroat trout.

Behavior	Description
Charge	Direct, rapid movement towards another fish, often followed by a chase or a nip
Chase	Continued pursuit of another fish, often preempted by a display, charge, or nip, resulting in the displacement of the fish from the territory
Nip	A biting motion, often directed at the fins or gills of the targeted fish, usually preempted by or following a chase
Lateral Display	Erect fins, fish becomes tense and rigid, mouth slightly open, head higher than tail; often occurred between two fish oriented head to tail, usually slowly circling each other
Dorsal Display	Fish approached another fish head first, coming in with erect dorsal and pectoral fins, usually with head slightly lower than tail

Predator Avoidance: Predator avoidance was directly measured only with rainbow trout because predators were not trained to live feed during the westslope cutthroat trout assessment period. Experiments were performed using two 2778 L circular tanks. Each tank had overhead cover made from black plastic material over half of the tank. Three units of submerged cover, made from four 92 x 7.5 cm-frayed black plastic strips and anchored similarly to the submerged cover of the ENR treatment tanks, were placed in each tank. Water flow in each tank was set to 15 L/min. Eight brown trout from Saratoga National Fish Hatchery were trained to live feed (juvenile rainbow and cutthroat trout; 40-70 mm in length) for six months prior to the predator avoidance trials. Brown trout were between 493 mm and 468 mm at the time of the trials. One brown trout was placed in each tank and allowed to acclimate for 24 hours. After the acclimation period, 10 fish of similar size (60 ± 10 mm) from a single treatment tank were placed into the tank with the predator. After ten minutes, the test fish were retrieved from the predator tank and the number remaining were counted. The predators remained in the test tanks during the entirety of the trials. A total of 24 trials were performed, two from each treatment tank.

Stress Response

Hatchery-reared fish may have reduced stress response and altered disease resistance when compared to wild fish (Woodward and Strange 1987; Anderson 1990; Werner et al. 2006). Blood plasma cortisol, glucose, and lactate concentrations were measured before (time: 0) and after (time: 30 minutes) exposure of test fish to a stressor. At the end of the rearing trials, blood was collected from the caudal vasculature of eight randomly selected fish from each test tank using ½ cc syringes for pre-stress (time: 0) data. Once the fish were removed, blood samples were collected within 5 minutes of tank removal to ensure basal concentrations were measured. Sampled fish were euthanized with an overdose of MS-222. Following the collection of blood from unstressed fish, the remaining fish in each tank were netted and exposed to air for one minute. Blood was then collected from another eight randomly selected fish in each test tank 30 minutes following the stress exposure. Pooled blood was centrifuged for 5 minutes and the plasma split into two samples and either frozen for later analysis for cortisol concentration, or refrigerated and later sampled for glucose and lactate concentrations (cutthroat trout only). Blood plasma cortisol was determined by radioimmunoassay (Foster and Dunn 1974) as modified by Redding et al. (1984). The minimum detection limit was 0.2 ng/mL. Plasma glucose and lactate were measured using a VITROS DT60II blood chemistry dry slide analyzer. Ten µl of plasma was analyzed for each parameter. Detection limits were 20-450 mg/dL for glucose and 0.50-12.00 mmol/L for lactate. Glucose and lactate analysis was not performed on the rainbow trout because no significant response was found in the cutthroat trout analysis.

Proximate Analysis

Lipid and protein content among rearing treatments was measured and compared to determine if differences in body composition occurred among test fish. At the end of all trials and measurements, fifty fish from each tank were euthanized by an overdose of MS-222 and frozen for later analysis. The fifty fish were ground together to create a pooled sample for each tank. Samples were then analyzed for lipid content according to AOCS (2009a) method Am 5-04 and protein content according to AOCS (2009b) method Ba 4e-93 in the lab at the BFTC.

Data Analysis

A two-way analysis of variance (ANOVA) with rearing treatment and species as independent variables was used to test for differences and interactions in growth, condition, dorsal fin index, stress (cortisol) response, and lipid and protein content. Stress responses in glucose and lactate were analyzed using one-way ANOVA, as they were measured among the cutthroat trout rearing treatments only. Multiple comparisons were tested for significance using Tukey's honest significant difference. Generalized linear models were used to test for differences in the remaining variables (survival, behavior assessments, and coloration) using rearing treatment and species as the explanatory variables. Binomial logistic regression was used on the data from survival (0

= mortality, 1 = survival), proportion of nips to threats and displays (0 = threat/display, 1 = nip), and predator avoidance (0 = consumed by predator, 1 = survived trial), because of the binomial distribution of the data. Data from the cover use and territoriality (total aggression) was measured as counts. Count variables were analyzed with Poisson regression. Proportional odds logistic regression was used on the coloration data from each species, because the data was in the form of ordered categorical responses. Likelihood ratio tests, as approximated by χ^2 values, were used to compare the models tested to a model with no predictors. Each coefficient from the logistic regression models was assessed for statistical significance using a Wald test, as estimated by a *Z* statistic, except for the proportional odds logistic regression, in which the coefficients were assessed by extra sum of squares *F*-tests, as approximated by *t*-statistics. The reference level in all generalized linear models was the westslope cutthroat trout CON. Discriminant function analysis (DFA) using multiple response variables was performed to determine the degree of differences among the treatment groups. Response variables included survival, length, weight, condition, dorsal fin index, aggression, cover use, lipid content, protein content, and cortisol response. An initial multivariate analysis of variance (MANOVA) was applied to determine if centroid differences were present among the rearing treatments. The discriminant function model was assessed for goodness of fit by Wilks' lambda, as approximated by χ^2 statistics. The variables were tested for significance by the stepwise method, which compared models with and without each variable using an *F*-test. The stepwise analysis was guided by an *F*-value of 3.84 to enter and 2.71 to remove, as these values approximated *p*-values of 0.05 and 0.10

respectively. Pairwise comparisons of the rearing treatments in the discriminant function space were performed using an *F*-test based on Mahalanobis distances of group means. Jackknifed predictions were used to assess the classification accuracy of the DFA model. For all analyses, statistical significance was set at $\alpha = 0.05$. Statistical methods were derived from Ramsey and Schafer (2002). All statistical analyses were conducted using R version 2.11.1, with the exception of the DFA, which was analyzed using SPSS Statistics version 17.0.

Effects of Hatchery Rearing Environment on Outplant Survival

Rearing Facility and Study Population

Fish rearing for the outplant assessment took place at Sekokini Springs Natural Rearing Facility located north of Columbia Falls, Montana near the confluence of the North and Middle Forks of the Flathead River. The facility is a former private rainbow trout hatchery that has recently been purchased and renovated by MFWP for the development of a westslope cutthroat trout conservation hatchery. Water is delivered to the hatchery via four springs, providing water year-round varying from 5 to 18 °C. The study population used was westslope cutthroat trout attained from Washoe Park Trout Hatchery. Approximately 10,000 westslope cutthroat trout embryos were incubated in up-welling incubation jars until swim up, at which point they were randomly assigned to the treatment tanks.

Hatchery Rearing

Fish were reared in the three rearing environments as described above with two exceptions. The rearing tanks were larger and, because of space limitations, tanks were stacked such that the lower level of tanks was 0.9 m off the ground and the upper level was 1.9 m off the ground. The tanks on the lower level were restricted to the ENR treatments because of the plumbing required for the submerged feed delivery system. Nine tanks were used, with three replicates of each treatment. Eight of the nine tanks were 372 L (305 x 53 x 23 cm) fiberglass troughs. The remaining tank was a 465 L (305 x 61 x 25 cm) fiberglass trough and was used because of the inability to attain one of the other tanks. The outlet screen on the larger tank was moved forward making the available water volume approximately equal to the other eight tanks. Flows were set at 7.5 L/min for each tank. Initial density was 900 fish per tank (approximately 2.4 fish/L), which was lower than the density used at the BFTC rearing trial. The lower densities at Sekokini Springs were attributable to not having enough fry available to match the densities used at the BFTC. Water temperatures averaged 13 °C over the rearing period. Feeding schedules, feed amount, and feed type were the same as those used at the BFTC. Fish were placed into the treatment tanks on 17 June 2010 and reared in the treatment tanks for 60 days (18 August 2010), at which point fish were outplanted into natural rearing ponds.

Performance Assessments

Survival, length, weight, and condition were measured in the same manner as described above at 30 and 60 days. At the end of the 60 day rearing period, dorsal fin index was assessed on 30 fish from each tank and a sample of 50 fish from each tank was collected and frozen for proximate analysis. Dorsal fin index and proximate analysis of lipid and protein content were performed using the same methods as described above.

Rearing Ponds

Six rearing ponds were used to simulate an outplanting event and to assess growth and survival of the fish in a quasi-natural environment. The rearing ponds were located at the Crystal Lakes Hatchery facility near Fortine, MT. The ponds varied in size, shape, and cover availability. Two large linear ponds were split in half using barriers to create four separate rearing ponds. The barriers were created by burying anchored 3.2 mm seine netting along the bottom of the ponds and attaching the seine to wooden boards running across the ponds approximately 46 cm above the water surface. The boards were held in place using rebar posts buried in the bottom of the ponds and drilled into the wood. Two barriers were placed in each linear pond about 61 cm apart in an attempt to ensure no movement between the barriers. Prior to outplanting, estimates of the volumes of the six rearing ponds were made by taking length, weight, and depth transects (Table 4). These volume estimates were used to identify how many fish to place in each pond such that fish densities were similar in all rearing ponds. Each pond was then randomly assigned to hold fish from one of the three treatment groups, each treatment having two replicates. At the end of hatchery rearing, fish from within a treatment were randomly netted and combined into a holding container.

Table 4: Dimensions, cover availability, and number of fish stocked in the rearing ponds used for outplant assessment of westslope cutthroat trout reared in different hatchery rearing environments.

Pond Name	Treatment	Length (m)	Width (m)	Depth (m)	Volume (L)	# Fish	Fish / L
Northeast	ENR	23.8	7.6	0.5	82,825	234	0.002825
Northwest	CON	23.8	7.6	0.8	138,041	309	0.002825
Southeast	INT	23.8	6.1	0.5	66,260	187	0.002822
Southwest	ENR	23.8	6.1	0.8	110,433	312	0.002825
Square	CON	14.6	13.1	0.6	116,889	330	0.002823
Golf Course	INT	53.3	10.3	1.0	542,340	1532	0.002825

Fish were then counted out of the approximately 105 km from the hatchery. The water temperature of the tank trailer was slowly raised (approximately 1 ºC every 10 minutes) to within 1 ºC of the rearing ponds, at which point the fish were netted out and released into the pond. The fish were not fed and had no artificial predator protection during the outplant period. Water chemistry and food item availability were measured at day 0, 30, and 60 post-outplanting. Temperature data were collected from the ponds using HOBO® Water Temp Pro V2 thermographs. The thermographs were placed in three of the ponds (golf course, northwest, and southwest) on 30 August, 2010. The northwest pond shared the same water source as the northeast and square ponds, and the southwest pond shared the same water source as the southeast pond, thus, I assumed that the three thermographs would track the water temperature for all of the ponds. Water chemistry was measured using a YSI® Handheld Multiparameter Instrument. The instrument measured temperature, conductivity, dissolved oxygen, pH, and nitrates. Invertebrate and plankton availability was sampled via plankton tows. A Turtox® Tow Net with a 30.5 cm mouth and 243 μm mesh was used for the plankton tows. Two 3.05 m tows were performed just above the benthic vegetation in each pond at day 0, 30, and 60 post-outplanting. The volume of water sampled in the plankton tows was calculated as the length of the tow multiplied by the area of the mouth. The plankton collections were placed in ethanol for later analysis. In the lab, the detritus material and debris were removed from the samples, which were then weighed in order to estimate an invertebrate and plankton density.

At the end of a 60-day rearing period (23-24 October, 2010), fish were removed from ponds by a combination of seining and backpack electrofishing. Seine netting was attempted as the sampling method, however dense aquatic vegetation and the soft bottoms of the ponds made sampling difficult. Thus, multiple electrofishing passes were performed until no fish were captured, with one exception. The deep, golf course pond had dense aquatic vegetation mats such that fish could not float to the surface during electrofishing passes; because of this a two-pass depletion estimate was made to estimate the number of fish surviving at the end of the pond rearing period.

Outplant Assessments

Survival was assessed by counting the number of fish remaining in each pond at the end of the outplant rearing period. Survival of each treatment group was identified as the proportion of fish surviving at the end of the pond rearing period compared to the total number of fish released into the pond. Length and weight measurements were taken twice during the pond rearing period, once at 30 days and again at 60 days. Fifty fish were measured at the 30 day point and all fish captured were measured at the end of the rearing period, except for fish from the golf course pond where a subsample of 270 fish were measured. Fulton's condition factor (K) was assessed using the methods described above. Dorsal fin index was measured on 50 fish from each pond at the end of the outplant rearing period. Upon removal and measurement, a sample of 25-30 fish from each pond was frozen for measurement of lipid and protein content. Dorsal fin index and proximate analysis measurements were performed using the methods described above.

Data Analysis

A one-way ANOVA was used to test for differences in fish length, weight, condition, dorsal fin index, and lipid and protein content among the rearing treatments at the end of the hatchery rearing phase. Survival in the hatchery was analyzed using the binomial logistic regression methods described above. One-way ANOVA was also used to test for differences in water temperature, water chemistry, and food availability among the rearing ponds. For the significant one-way ANOVA, multiple comparisons were tested for significance using Tukey's honest significant difference test. Survival, growth, and lipid content at the end of the hatchery rearing phase could not be statistically evaluated because of a lack of replication.

RESULTS

Effects of Hatchery Rearing Environment on Performance

Survival

Survival of both species was high among all treatments, varying from 88.7 to 96.6% (Table 5). However, small but significant differences existed in survival among the rearing treatments (χ^2_5 = 95.45, $p < 0.01$). The survival of fish from the ENR (88.7%, $p < 0.01$) and INT (94.2%, $p = 0.03$) rearing treatments were significantly lower than the CON (96.0%) treatment. The lower ENR survival stemmed from an abnormally high number of mortalities (n=68) in one cutthroat trout ENR tank compared to fewer than 30 mortalities in all other tanks. Survival did not differ significantly between species (p = 0.86), although the interaction between rainbow trout and the ENR treatment was significant, having 7.9% higher survival compared to the cutthroat trout ENR treatment ($p < 0.01$).

Table 5: Survival, standard errors, and associated *p*-values of westslope cutthroat trout (WCT) and rainbow trout (RBT) from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from binomial logistic regression. Asterisks indicate variables significantly different from the reference level.

Variable	Survival (%)	S.E.	*p*-Value
WCT-CON	96.0	0.14	
WCT-INT	94.2	0.17	0.03*
WCT-ENR	88.7	0.18	< 0.01*
RBT-CON	95.9	0.19	0.86
RBT-INT	95.8	0.26	0.15
RBT-ENR	96.6	0.26	< 0.01*

Growth

Fish length and weight at the end of the hatchery rearing period varied from 56.7 to 62.5 mm and from 2.0 to 2.8 g. At the 30-day interval, length and weight were not significantly different among the rearing treatments (length: $F_{2, 20} = 2.50$, $p = 0.11$; weight: $F_{2, 20} = 3.04$, $p = 0.07$; Figures 2 and 3). However, there were significant differences between species (length: $F_{1, 20} = 65.36$, $p < 0.01$; weight: $F_{1, 20} = 65.72$, $p < 0.01$), with rainbow trout averaging 4.3 mm-longer and 0.3 g-heavier (9.8% and 35.6% respectively) than cutthroat trout after 30 days.

Length at the end of the 60-day hatchery rearing period was significantly different among rearing treatments and between species (treatments: $F_{2, 20} = 8.95$, $p < 0.01$; species: $F_{1, 20} = 7.70$, $p = 0.01$; Figures 4 and 5). Final weight was also significantly

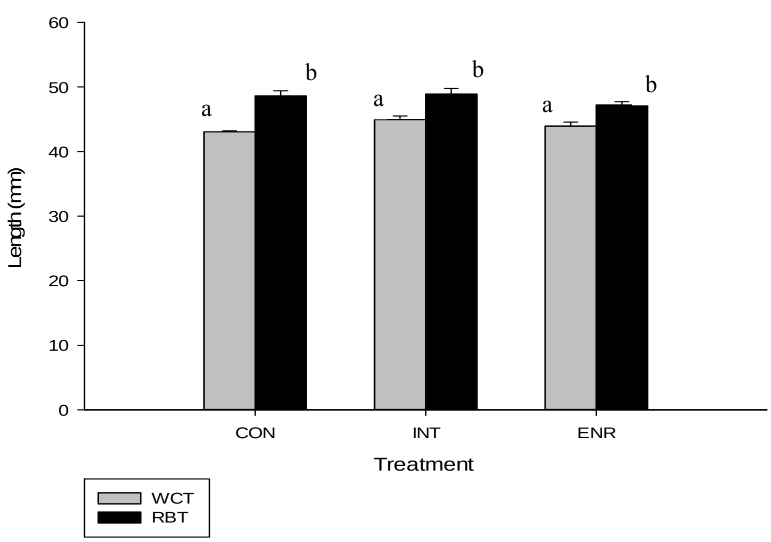


Figure 2: Mean length (mm) (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) after 30 days in three different hatchery rearing environments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR). Different letters indicate significant differences ($p < 0.05$; Tukey's multiple comparisons).

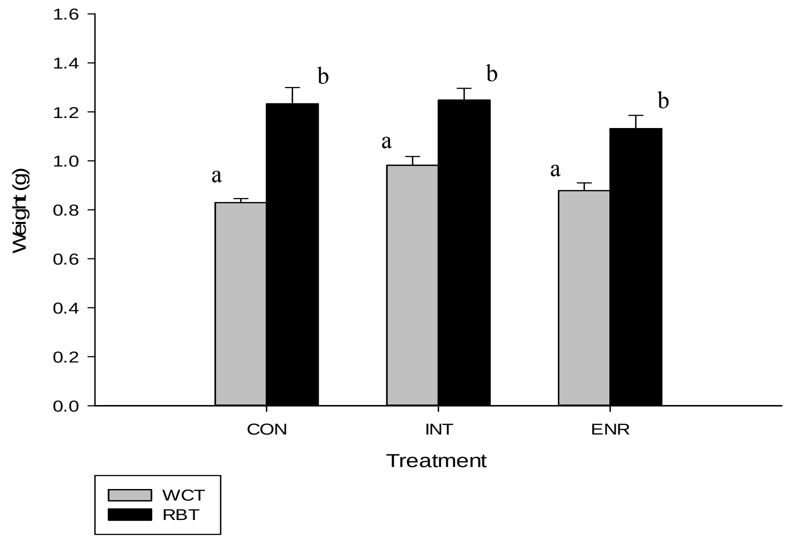


Figure 3: Mean weight (g) (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) after 30 days in three different hatchery rearing environments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

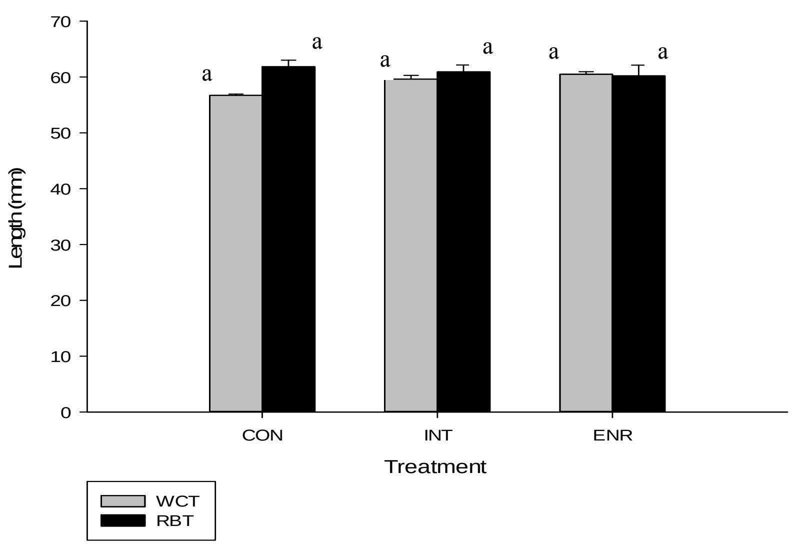


Figure 4: Mean length (mm) (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) after 60 days in three different hatchery rearing environments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

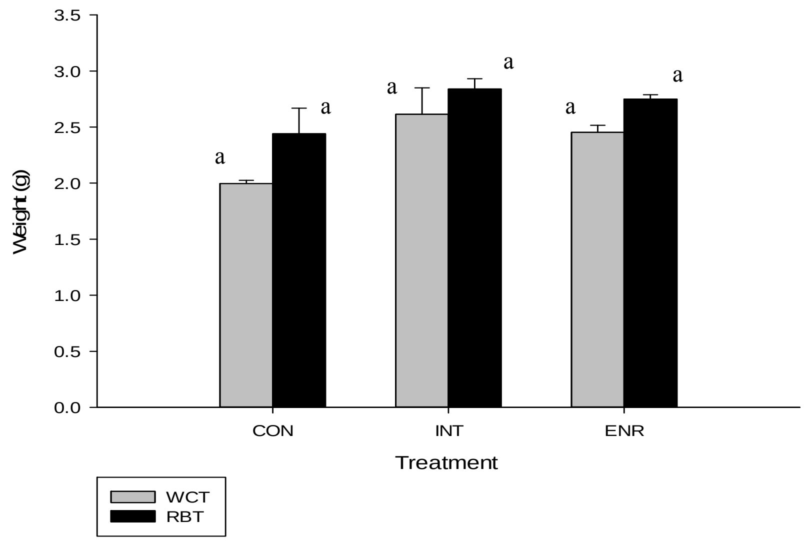


Figure 5: Mean weight (g) (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) after 60 days in three different hatchery rearing environments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

different among the treatments and between species (treatments: $F_{2, 20} = 7.38$, $p < 0.01$; species: $F_{1, 20} = 8.14$, $p = 0.01$). Length and weight in the INT (length: $p < 0.01$; weight: $p < 0.01$) and ENR treatments (length: $p < 0.01$;weight: $p = 0.03$) was significantly higher than the length and weight of the CON treatment after 60 days. The INT treatment was 3.3 mm (5.8%) longer and 0.5 g (23.8%) heavier and the ENR treatment was 3.3 mm (5.8%) longer and 0.4 g (19.0%) heavier than the CON treatment. Length and weight in the INT and ENR treatments did not differ significantly ($p > 0.05$). Rainbow trout averaged 3.5%-longer (2.0 mm; $p = 0.01$) and 14.3%-heavier (0.3 g; $p = 0.01$) than cutthroat trout at the end of the rearing trials.

Condition

Body condition at the end of the rearing period varied from 1.09 to 1.23, and did not differ among rearing treatments or between species (treatment: $F_{2,20} = 0.97$, $p = 0.40$; species: $F_{1,20} = 0.62$, $p = 0.44$; Figure 6).

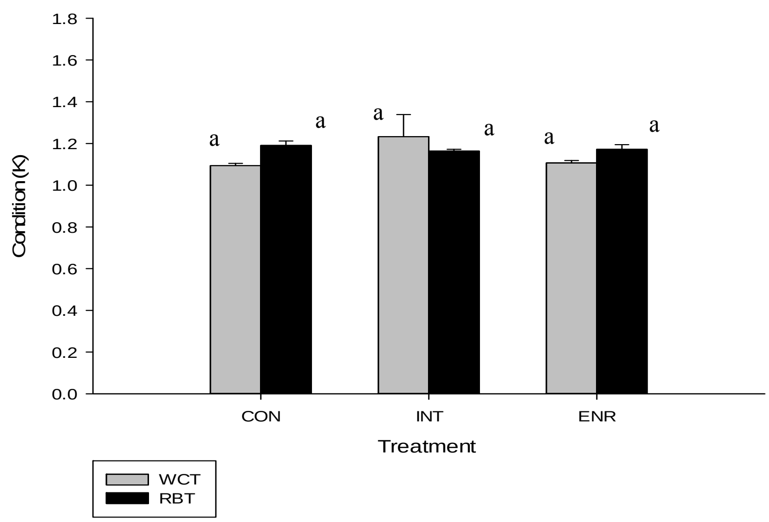


Figure 6: Mean Fulton's condition factor (K) (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) after 60 days in three different hatchery rearing environments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

Dorsal Fin Index

The dorsal fin index differed significantly among rearing treatments ($F_{2,18} = 102.14$, $p < 0.01$; Figure 7) and species ($F_{1,18} = 52.64$, $p < 0.01$). The dorsal fin index of the ENR treatment (11.4) was significantly higher than the CON (8.3; $p < 0.01$) and INT (9.7; $p < 0.01$) treatments. Rainbow trout dorsal fin index averaged 1.3 higher than cutthroat trout ($p < 0.01$). The interaction between rearing treatment and species was also significant ($F_{2,18} = 12.34$, $p < 0.01$), with rainbow trout having significantly higher dorsal fin index in the INT ($p < 0.01$) and ENR treatments ($p = 0.05$) than did the cutthroat trout.

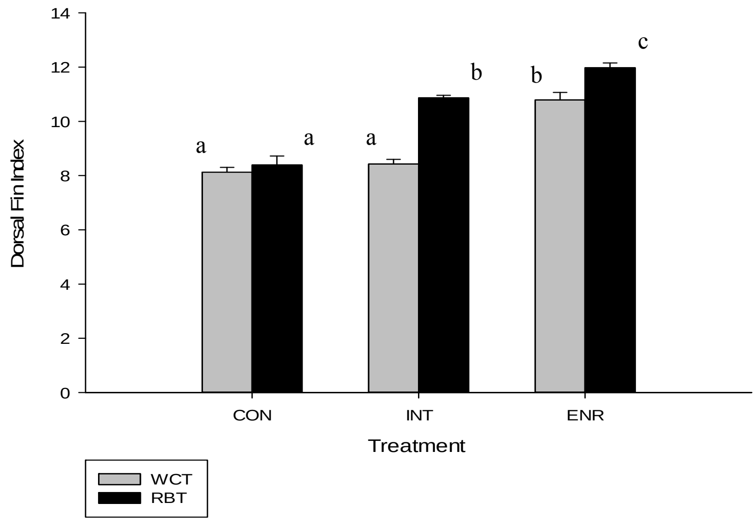
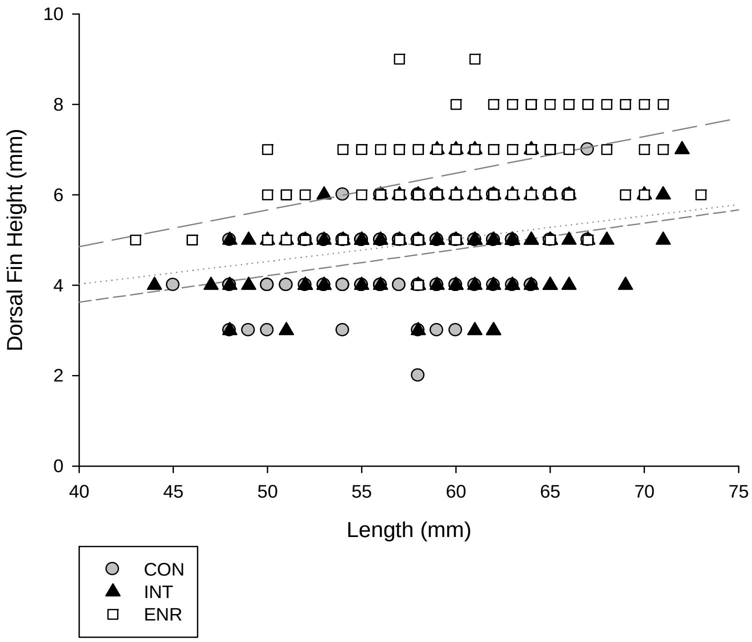
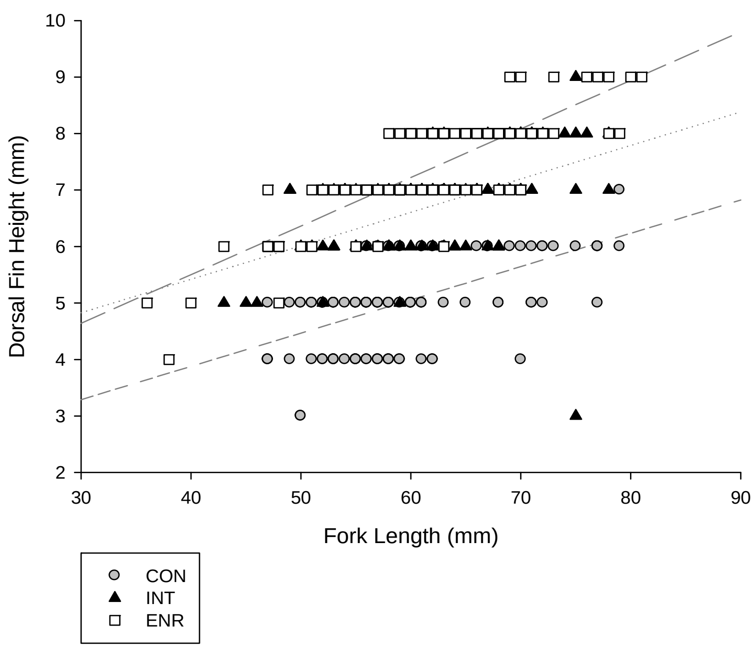


Figure 7: Mean dorsal fin index (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) at 60 days from three different hatchery rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

Regression lines of each treatment fitted to a scatter-plot of dorsal fin height and fork length was assessed to determine differences in dorsal fin height at a given fork length. A cutthroat trout of 70 mm had an average dorsal fin height of 5.4 mm in the CON treatment, 5.5 mm in the INT treatment, and 7.3 mm in the ENR treatment (Figure 8). For a rainbow trout of 70 mm, the average dorsal fin height was 5.7 mm in the CON treatment, 7.2 mm in the INT treatment, and 8.2 mm in the ENR treatment (Figure 8).



a)



b)

Figure 8: Scatterplot and regression lines of length by dorsal fin height for a) westslope cutthroat trout and b) rainbow trout from three different hatchery rearing treatments: conventional (CON: short-dashed line), enriched-intermediate (INT: dotted line), and enriched-high (ENR: long-dashed line).

Coloration

Coloration differed significantly among rearing treatments (χ^2_5 = 564.60, $p <$ 0.01), with fish from CON treatment tanks showing a significantly lower coloration scores than fish from the ENR tanks (Table 6). For cutthroat trout, 35.9% of fish from the ENR treatment had dark green coloration and well developed spots and parr marks (categories 4 and 5), whereas only 17.5% of CON fish and 7.5% of INT fish had high coloration scores. Similar findings occurred with rainbow trout, as 32.4% of ENR fish and only 12.8% of CON fish had high coloration scores, although the percentage of high coloration scores in the INT treatment increased drastically among rainbow trout (44.8%). Coloration scores between the CON and INT treatments did not differ (p = 0.35), however the ENR fish were 4.3 times more likely to be placed in a higher coloration category than CON fish ($p < 0.01$). Coloration scores did not differ between the species among the CON (p = 0.47) and ENR treatments (p = 0.47), although rainbow trout had significantly higher coloration scores in the INT treatment ($p < 0.01$). Rainbow trout from the INT treatment were 21.4 times more likely to be placed in a higher coloration category than cutthroat trout from the INT treatment.

Table 6: Odds of higher coloration score compared to reference level, standard errors, and associated *p*-values of westslope cutthroat trout (WCT) and rainbow trout (RBT) from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from proportional odds logistic regression. Asterisks indicate variables significantly different from the reference level.

Variable	Odds	S.E.	*p*-Value
WCT-CON	1	0.34	
WCT-INT	1.2	0.42	0.35
WCT-ENR	4.3	0.44	< 0.01*
RBT-CON	0.9	0.43	0.47
RBT-INT	21.3	0.61	<0.01*
RBT-ENR	3.8	0.60	0.48

Behavior

Fish behavior was noticeably different among the rearing treatments during the 60-day trials. At 2-3 weeks after the start of the trial, fish from the CON treatment appeared to become conditioned and associated humans with food. This was prominently displayed whenever a human passed by the rearing tanks, as these fish would aggregate at the surface near the front of the tank where food was added. In contrast, fish from the INT and ENR rearing treatments displayed a fright response to human disturbance during daily cleaning and feeding. Fish distribution also varied among treatments. Fish from the CON treatment tended to remain aggregated at the outlet end of the tank near the water surface, whereas fish from the INT treatment aggregated under cover near the belt feeder, and fish from the ENR treatment remained fairly evenly distributed throughout the tank and tended to be found near the bottom (Figure 9). These behavioral differences among treatments were consistent for both rainbow trout and westslope cutthroat trout.

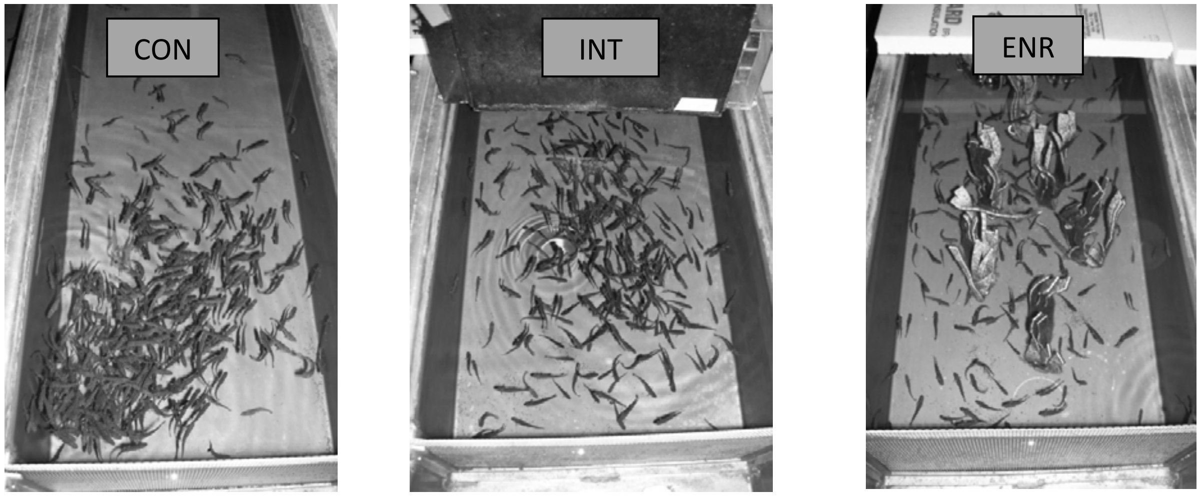
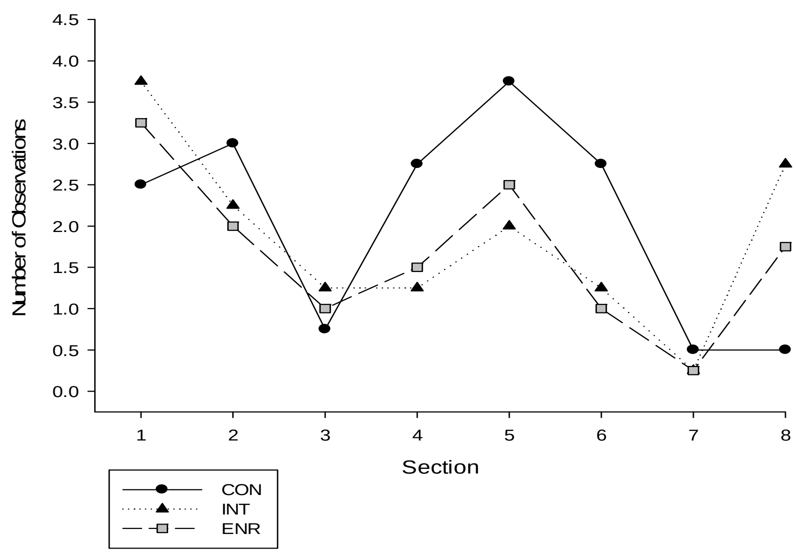


Figure 9: Typical distribution in hatchery rearing tanks of rainbow trout and westslope cutthroat trout from three different rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

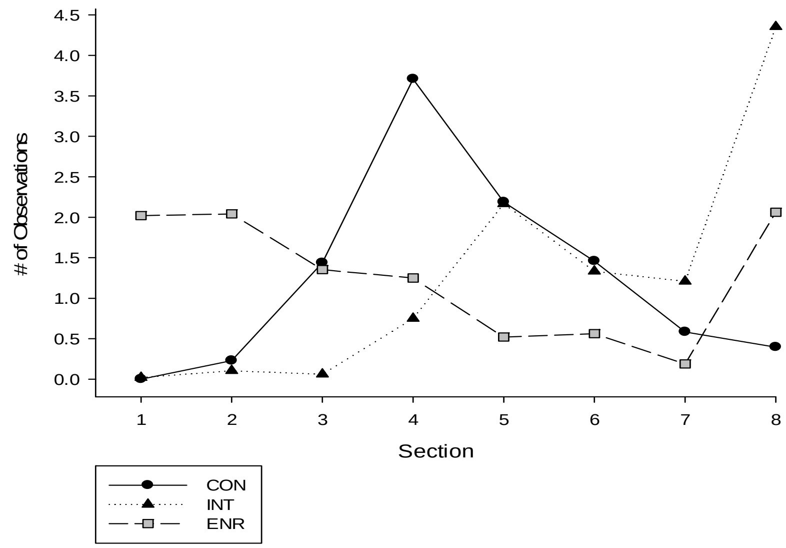
Cover: During the cover trials, fish showed an initial fright response after placement into the test tank that usually subsided within the first 30 seconds of observation. Fish from the INT and ENR treatments were more likely to be found under cover than fish from the CON treatment in both species (Figure 11; $= 55.47$, $p < 0.01$; Table 7). The odds of cover use in the INT and ENR treatments were 3.0 ($p < 0.01$) and 1.6 ($p = 0.02$) times greater than the odds of cover use in the CON treatment. The distribution of cutthroat trout in the cover tank was similar among the rearing treatments, except for the covered section of the tank, while the distribution of rainbow trout throughout the test tank was different among the rearing treatments (Figure 10). The apparent preference for the first two sections of the tank can mostly be explained by fish that did not move about the tank during the observation period, likely because of fright response. Fish from the CON rearing treatment tended to remain in the middle of the tank and largely avoided the covered section. The INT and ENR treatments were found at the ends of the tank the majority of the time, often under cover or near the first two sections of the tank.

Table 7: Odds of cover use, standard errors, and associated *p*-values of westslope cutthroat trout (WCT) and rainbow trout (RBT) from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from Poisson logistic regression. Interactions were not significant. Asterisks indicate variables significantly different from the reference level.

Variable	Odds	S.E.	*p*-Value
WCT-CON	0.3	0.53	
WCT-INT	3.0	0.56	<0.01*
WCT-ENR	1.6	0.53	0.02*
RBT	0.4	0.29	0.25



a)



b)

Figure 10: Distribution of a) westslope cutthroat trout and b) rainbow trout from different hatchery rearing treatments in experimental cover seeking tank. Treatments include conventional (CON), enriched-intermediate (INT), and enriched-high (ENR). Section 8 was the covered portion of the tank.

<u>Territoriality</u>: In most trials, fish had established social hierarchies, dominance, and began feeding by the start of the first observation period. Dominant fish established territories at the head of the pool, and submissive fish were commonly found at the rear of the pool or occasionally at the head of the riffle. Feeding was done predominantly by the dominant fish during each trial.

Aggression varied significantly among rearing treatments and species (χ^2_3 = 752.87, $p < 0.01$). The number of aggressive acts by rainbow trout and cutthroat trout from the CON treatment were nearly double the number of aggressive acts in the INT and ENR treatments (Table 8). The odds of an aggressive act in the INT and ENR treatments were 48.3% ($p < 0.01$) and 48.1% ($p < 0.01$) less than in the CON treatment. Rainbow trout exhibited significantly more aggressive acts (65.4%) across all treatments than did cutthroat trout ($p < 0.01$).

Table 8: Odds of an aggressive act and nip attacks compared to reference level, standard errors, and associated *p*-values of westslope cutthroat trout (WCT) and rainbow trout (RBT) from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from Poisson logistic regression. Interactions were not significant. Asterisks indicate variables significantly different from the reference level.

Variable	Aggressive Acts			Nip Attacks		
	Odds	S.E.	*p*-value	Odds	S.E.	*p*-value
WCT-CON	1	0.03		1	0.06	
WCT-INT	0.52	0.04	<0.01*	0.65	0.08	<0.01*
WCT-ENR	0.52	0.04	<0.01*	0.73	0.07	<0.01*
RBT	1.65	0.03	<0.01*	1.98	0.06	<0.01*

The type of aggressive acts also differed (Table 8), as the prevalence of nips differed significantly among the rearing treatments and species ($\chi^2_3 = 150.57$, $p < 0.01$). Significantly more nips compared to threats and displays occurred in the CON treatment than in the INT and ENR treatments, with nips being 44.7% ($p < 0.01$) and 37.3% less likely ($p < 0.01$) in each treatment. The odds of a nip in the rainbow trout trials were 2.0 times the odds in the cutthroat trout across all rearing treatments ($p < 0.01$).

<u>Predator Avoidance</u>: Upon placement into the predator avoidance tank, most rainbow trout introduced into the tank with a brown trout predator schooled together and remained near the edges of the tank. The predator was often under the submerged cover. At the end of the trial, rainbow trout were no longer schooled together, rather most were hiding in the submerged cover in small groups of three or fewer, whereas the brown trout

was commonly circling the tank and evidence of movement could be seen by displaced submerged cover.

Prey survival was generally high across all trials, averaging 80.0 to 92.5% (Table 9). Rearing treatment of rainbow trout did not have a significant effect on the odds of consumption by brown trout ($\chi^2_2 = 2.76$, $p = 0.25$).

Table 9: Predation survival rate of rainbow trout (RBT), standard errors, and associated *p*-values from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from binomial logistic regression.

Variable	Survival (%)	S.E.	*p*-Value
RBT-CON	87.5	.048	
RBT-INT	80.0	0.77	0.46
RBT-ENR	92.5	0.62	0.37

<u>Stress Response</u>

Basal cortisol concentrations did not differ among the rearing treatments ($F_{2, 20} = 0.12$, $p = 0.88$) or between species ($F_{1, 20} = 1.23$, $p = 0.28$). Basal cortisol concentrations averaged 4.23 ng/mL. There was no differences in post-stressor cortisol concentrations either (treatment: $F_{2, 20} = 0.63$, $p = 0.54$; species: $F_{1, 20} = 1.16$, $p = 0.29$; Figure 11). Post-stressor cortisol concentrations averaged 44.8 ng/mL in the CON treatment, 50.3 ng/mL in the INT treatment, and 53.0 ng/mL in the ENR treatment.

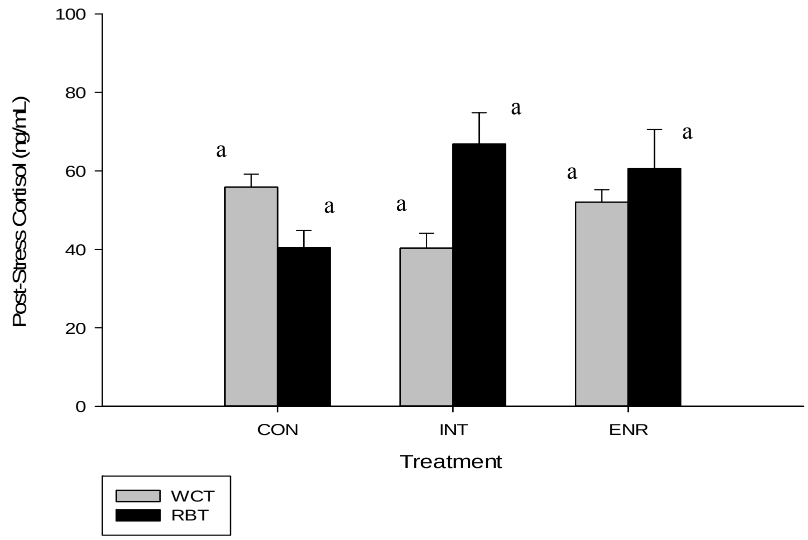


Figure 11: Post-stress concentrations of cortisol (ng/mL; + S.E.) 30 minutes after a 1 minute air exposure of westslope cutthroat trout (WCT) and rainbow trout (RBT) among three different rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

Basal concentrations of glucose (68.8 mg/dL) and lactate (1.3 mmol/L) did not differ among rearing treatments in cutthroat trout (glucose: $F_{2, 9} = 1.15$, $p = 0.36$; lactate: $F_{2, 9} = 1.15$, $p = 0.36$). There were significant differences in glucose concentrations among the rearing treatments post-stressor ($F_{2, 9} = 4.5$, $p = 0.04$), but not in lactate concentrations ($F_{2, 9} = 4.2$, $p = 0.05$). Stress response, as measured by glucose, was 15% and 11% less in the INT and ENR treatments than in the CON (Table 10). Post-stress lactate concentrations averaged 7.3 mmol/L among the rearing treatments.

Table 10: Mean (standard error) basal and 30 minute post-stress concentrations of glucose and lactate of westslope cutthroat trout among conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing treatments. Asterisks indicate statistically significant difference.

Treatment	Glucose (mg/dL)		Lactate (mmol/L)	
	Basal	Post-Stress	Basal	Post-Stress
CON	73.25 (4.2)	94.5 (3.0)	1.28 (0.2)	7.45 (0.5)
INT	64.25 (5.9)	82.25* (4.3)	1.13 (0.3)	6.22 (0.6)
ENR	69 (5.9)	85 (4.3)	1.63 (0.3)	8.05 (0.6)

Proximate Analysis

Lipid content was different among rearing treatments ($F_{2, 18} = 11.75$, $p < 0.01$; Figure 12), varying from 6.9 to 8.0%. Overall, lipid content of rainbow trout was

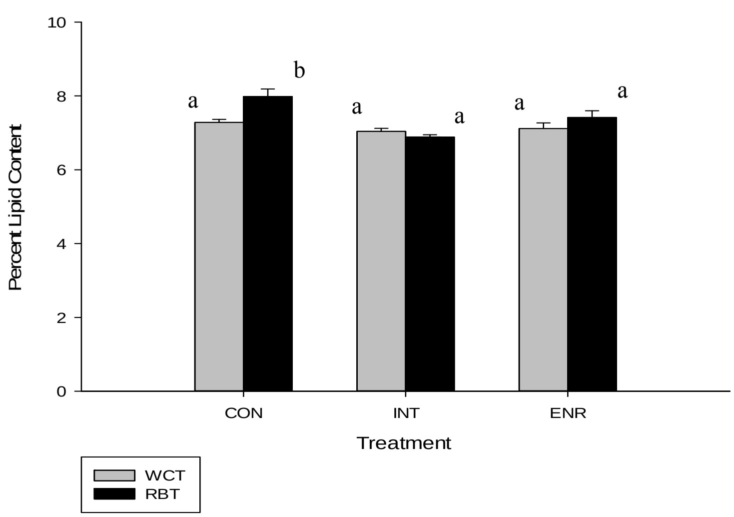


Figure 12: Mean percent lipid content (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) from three different hatchery rearing treatments: Conventional (CON), Enriched-intermediate (INT), and Enriched-high (ENR).

slightly (0.7%) but significantly higher than cutthroat trout ($F_{1, 18} = 6.18$, $p = 0.02$). The interaction between treatment and species was also significant ($F_{2, 18} = 4.69$, $p = 0.02$), with the rainbow trout INT treatment associated with a decrease in lipid content of 0.9%. Protein content did not differ among rearing treatments ($F_{2, 20} = 1.22$, $p = 0.32$) ranging from 15.1 to 15.6%, however differences between species existed ($F_{1, 20} = 15.06$, $p < 0.01$; Figure 13). Rainbow trout had significantly less protein content (0.4%) than cutthroat trout ($p < 0.01$).

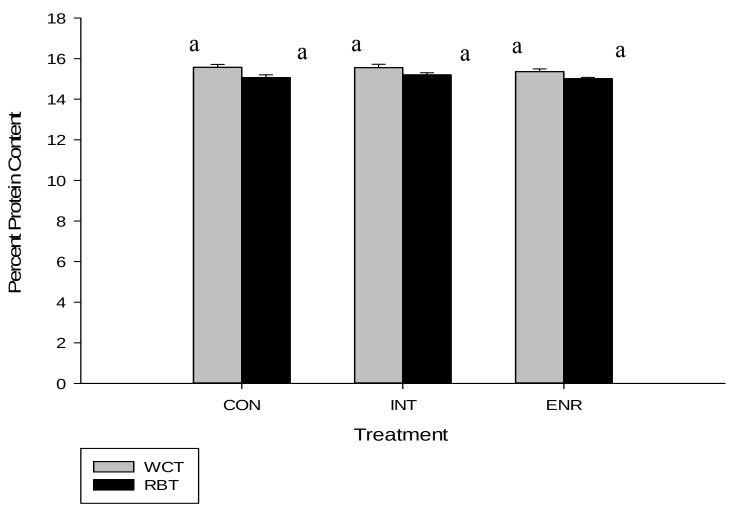
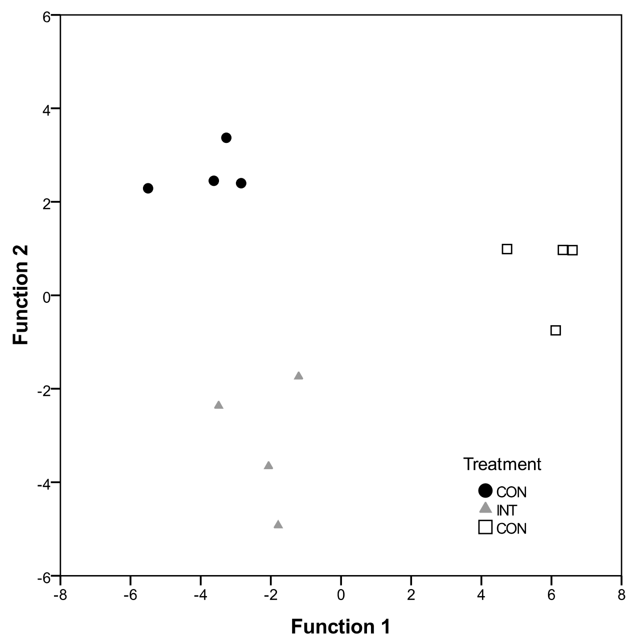


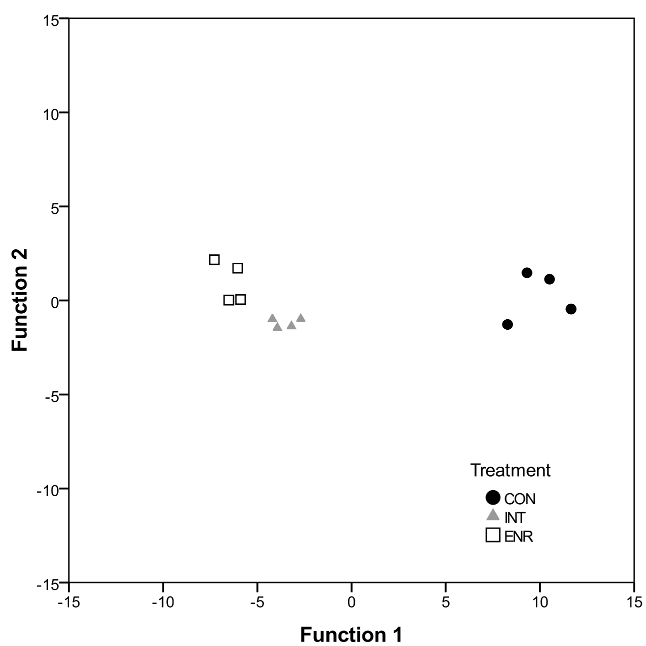
Figure 13: Mean percent protein content (+ S.E.) of westslope cutthroat trout (WCT) and rainbow trout (RBT) from three different hatchery rearing treatments: Conventional (CON), Enriched-intermediate (INT), and Enriched-high (ENR).

Multivariate Analysis

In westslope cutthroat trout ($F_{10, 12} = 10.52$, $p < 0.001$) and rainbow trout ($F_{10, 12} = 3.06$, $p = 0.043$), significant differences existed among the rearing treatments based centroid analysis (Figure 14). For cutthroat trout, the discriminant model was significant ($\chi_8^2 = 40.39$, $p < 0.01$) and dorsal fin index, length, lipid content, and cover were significant terms in the model ($p < 0.05$). The first discriminant function explained 75.9% of the variation and dorsal fin index, final length, and cover use contributed most to the function (Table 11). The second function accounted for 24.1% of the variation, with lipid content, final length, and cover use contributing to the function. The pairwise comparisons among the treatments along the discriminant function axes were significantly different for all comparisons (Table 12; $p < 0.05$). For rainbow trout, the discriminant model was significant ($\chi^2_6 = 39.75$, $p < 0.01$) and dorsal fin index, lipid content, and condition were significant to the analysis ($p < 0.05$). Dorsal fin index and condition contributed most to the first discriminant function (Table 11), which accounted for 98.4% of the variation. The second discriminant function, consisting primarily of lipid content, did not discriminate among the rearing treatments, accounting for only 1.6% of the variation. All pairwise comparisons of the rearing treatments along the discriminant function axes were significantly different (Table 12; $p < 0.05$). The DFA model predicted the correct treatment based on summary variables 91.7% of the time for cutthroat trout and 100% of the time for rainbow trout. The data used for the DFA can be found in appendix A.



a)



b)

Figure 14: Discriminant function plots of three rearing treatments for a) westslope cutthroat trout and b) rainbow trout. The conventional treatment (CON) is represented by black circles, the enriched-intermediate treatment (INT) is represented by grey triangles, and the enriched-high treatment (ENR) is represented by white squares.

Table 11: Summary of standardized canonical discriminant function coefficients among variables significant to discriminant function analysis models for westslope cutthroat trout (WCT) and rainbow trout (RBT).

Species	Variable	Discriminant Function 1	Discriminant Function 2
WCT	Final Length	0.93	-0.92
	Dorsal Fin Index	1.21	0.62
	Cover	-0.77	-0.81
	Lipid	0.03	1.39
RBT	Dorsal Fin Index	-1.78	0.29
	Lipid	0.73	0.89
	Condition	1.47	0.01

Table 12: *F*-statistics and *p*-values of all pairwise comparisons among rearing treatments in the discriminant function space for westslope cutthroat trout (WCT) and rainbow trout (RBT). Rearing treatments are conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

Species	Comparison	F-Statistic $_{(d.f.)}$	p-Value
WCT	CON – INT	$12.13_{(4, 6)}$	0.005
	CON – ENR	$33.17_{(4, 6)}$	<0.001
	INT – ENR	$26.38_{(4, 6)}$	0.001
RBT	CON – INT	$94.77_{(3, 7)}$	< 0.001
	CON – ENR	$139.27_{(3, 7)}$	< 0.001
	INT – ENR	$6.92_{(3, 7)}$	0.017

Effects of Hatchery Rearing Environment on Outplant Survival and Performance

General Observations

Most behaviors during the rearing period at the Sekokini hatchery were similar to those observed during trials at the BFTC. The one marked difference was in the human response of fish from the CON treatment. In the previous trials with rainbow trout and cutthroat trout, fish from the CON rearing treatment became trained to associate humans with food which led to a loss of fright response to humans. This was not the case during the Sekokini hatchery rearing trials. The response to humans in the CON treatment did not develop, possibly because of the larger tanks or the stacked design of the rearing treatments and therefore less human exposure.

Hatchery Performance

Survival of cutthroat trout was similar to that observed during rearing trials at the BFTC, as survival in all treatments was near 90% or higher (Table 13). Slight differences in survival among rearing treatments at Sekokini were statistically significant ($\chi_2^2 = 30.85$, $p < 0.01$). The mean survival rate in the ENR tanks was 89.8% ($p < 0.01$), 89.5% ($p < 0.01$) in the INT tanks, and 93.7% in the CON tanks.

Table 13: Hatchery survival rate, standard errors, and associated *p*-values of westslope cutthroat trout from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments as determined from binomial logistic regression. Asterisks indicate statistical differences to reference level.

Variable	Survival (%)	S.E.	*p*-Value
CON	94.1	0.08	
INT	90.5	0.11	<0.01*
ENR	90.7	0.10	<0.01*

Length and weight at the end of hatchery rearing varied from 49.5 to 55.8 mm and 1.4 to 1.9 g respectively (Figure 15). Length and weight were significantly lower in the ENR treatment at both 30 (length: $F_{2,6} = 16.99$, $p < 0.01$; weight: $F_{2,6} = 11.21$, $p = 0.01$) and 60 days (length: $F_{2,6} = 69.95$, $p < 0.01$; weight: $F_{2,6} = 45.26$, $p < 0.01$), averaging 6.2 mm and 0.4 g smaller than the CON and INT treatments at the end of the rearing period.

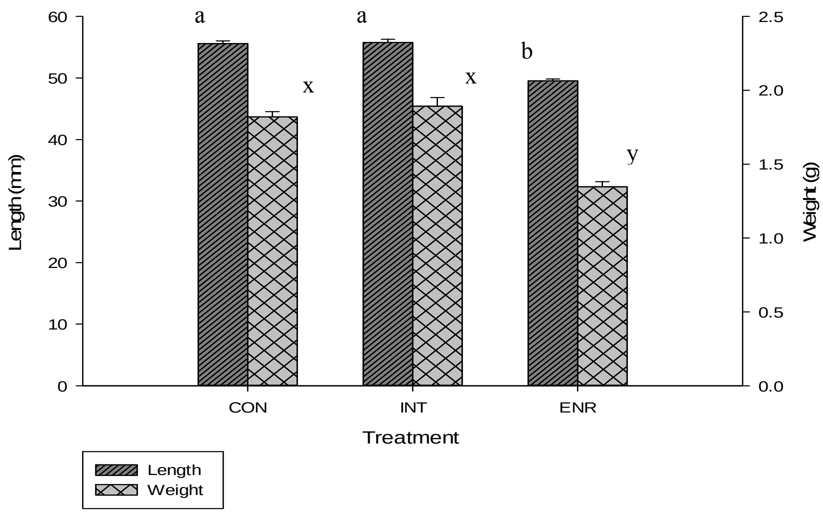


Figure 15: Mean length (mm) and weight (g) of westslope cutthroat trout (+ S.E.) at the end of a 60 day rearing period from three different hatchery rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR). Different letters indicate significant differences ($p < 0.05$; Tukey's multiple comparisons), the letters a, b, and c are used for the length comparisons and x, y, and z are used for the weight comparisons.

Fulton's condition factor (K) was not significantly different among the rearing treatments at day 60 ($F_{2, 6} = 3.562$, $p = 0.10$), with condition varying from 1.06 to 1.11.

Significant differences existed in the dorsal fin index among the rearing treatments ($F_{2, 6} = 21.81$, $p < 0.01$) at day 60. Mean dorsal fin index was significantly higher in the ENR treatment (11.0, $p < 0.01$) than in the INT (9.4) and CON (9.0) treatments (Figure 16).

Lipid and protein content did not differ among rearing treatments (Lipid: $F_{2, 6} = 4.43$, $p = 0.07$; Protein: $F_{2, 6} = 2.71$, $p = 0.15$), with lipid content varying from 6.1 to 6.8% and protein content varying from 13.9 to 14.2% (Figure 17).

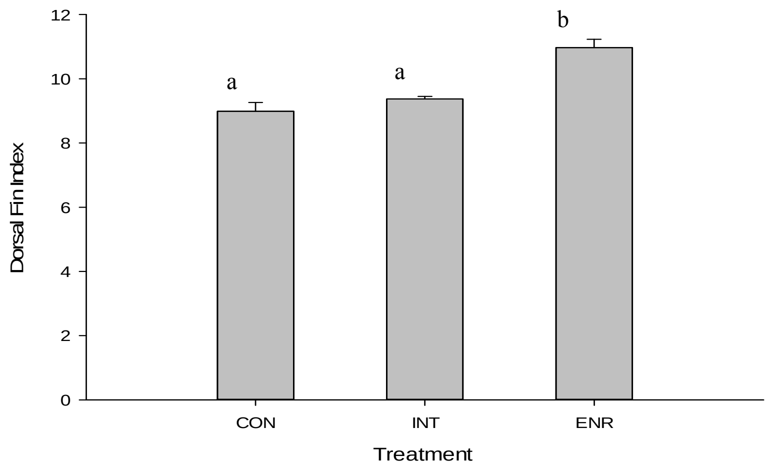


Figure 16: Mean dorsal fin index (+ S.E.) at the end of a 60 day rearing period of westslope cutthroat trout from three different hatchery rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR).

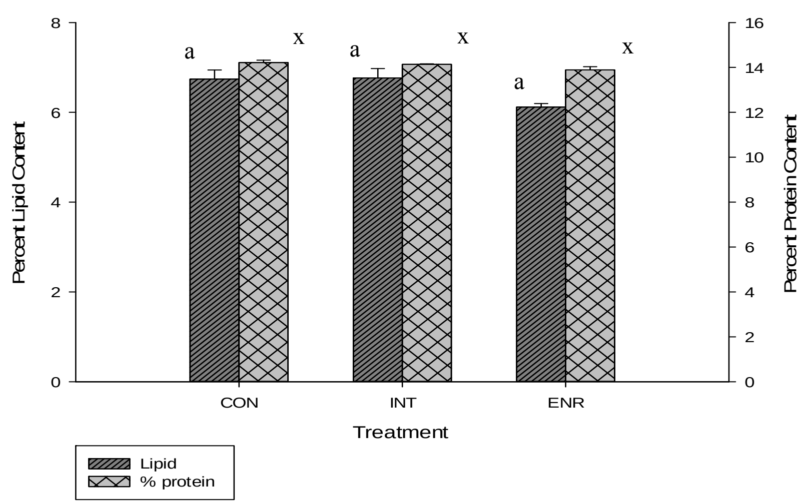
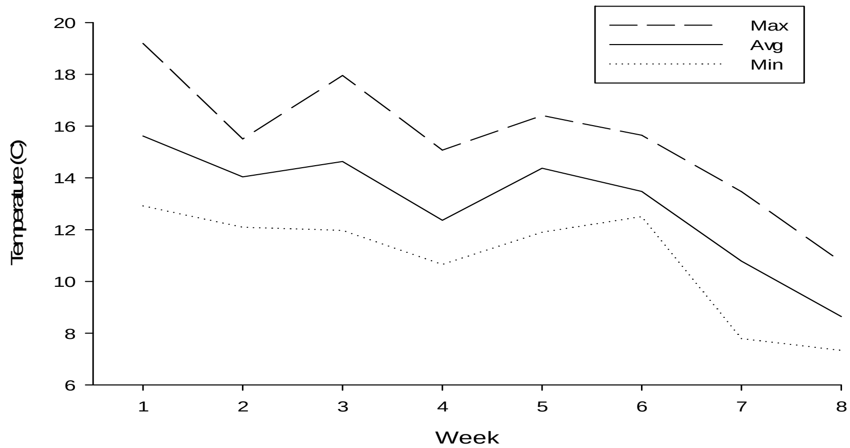


Figure 17: Mean percent lipid and protein content (+ S.E.) determined by proximate analysis of westslope cutthroat trout at the end of a 60 day rearing period among three different hatchery rearing treatments: conventional (CON), enriched-intermediate (INT), and enriched-high (ENR). Different letters indicate significant differences ($p < 0.05$; Tukey's multiple comparisons), the letters a, b, and c are used for the length comparisons and x, y, and z are used for the weight comparisons.

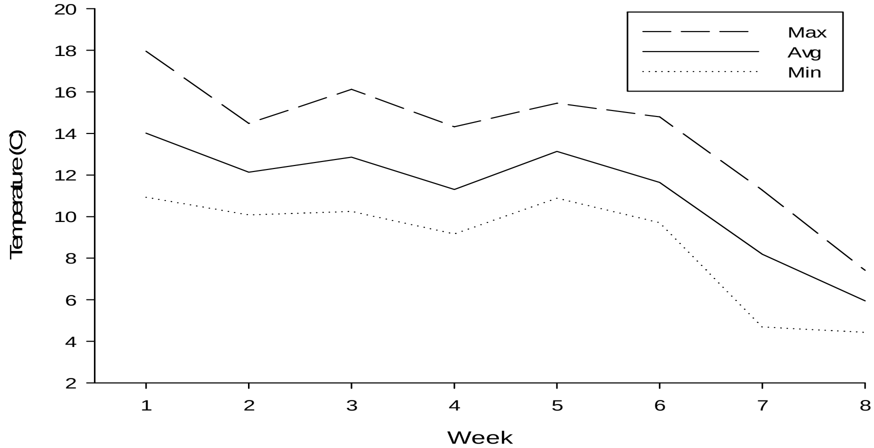
Outplant Performance

Water temperatures in the rearing ponds at the time of outplanting were 19-21.5 °C. Average daily and maximum water temperatures differed significantly among the rearing ponds (daily: $F_{2, 159} = 12.06$, $p < 0.01$; maximum: $F_{2, 159} = 7.53$, $p < 0.01$) averaging 12.9 and 14.1 °C in the golf course pond, 11.0 and 12.4 °C in the north hatchery pond and 10.6 and 12.1 °C in the south hatchery pond. The daily average and maximum water temperatures were significantly higher in the golf course pond than in the other ponds, averaging 1.9 ($p < 0.01$) and 2.3 °C ($p < 0.01$) warmer daily average temperatures than the northwest and southwest ponds and 1.7 ($p < 0.01$) and 2.0 ($p < 0.01$) °C warmer maximum temperatures (Figure 18). The daily and maximum temperatures did not differ significantly between the northwest and southwest thermographs.

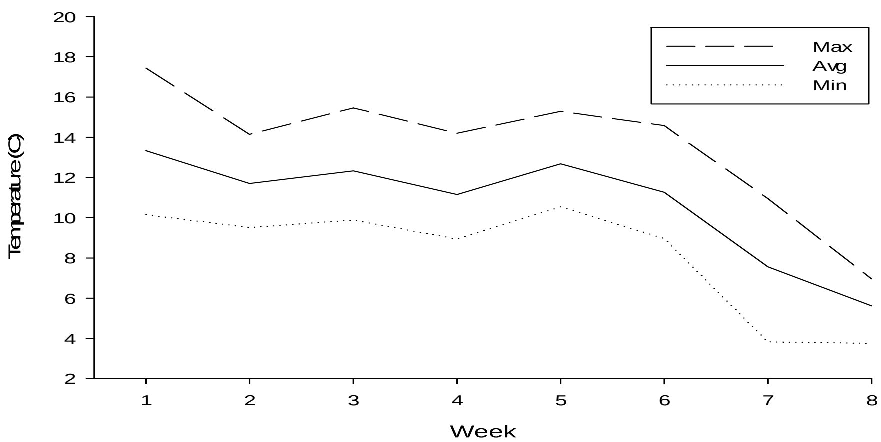
Water chemistry did not differ significantly among the rearing ponds ($p > 0.05$), with the exception of the golf course pond (Table 14). Conductivity (235.29 µS/cm) and dissolved oxygen (9.85 mg/L) were significantly higher ($p < 0.05$) in the golf course pond than in the other rearing ponds. Conductivity ranged from 115.1 to 293.5 µS/cm and dissolved oxygen from 7.5 to 10.5 mg/L during the pond-rearing period. Dissolved oxygen tended to increase during the pond-rearing period, likely because of lower water temperatures as summer transitioned into autumn (Figure 19). Nitrate levels at day 60 were not recorded because of improper calibration of the sampling unit. Nitrate levels increased from day 0 (0.3 mg/L) to 30 (0.5 mg/L) post-outplanting, however, the lack of day 60 data limited any detection of trends.



a)



b)



c)

Figure 18: Weekly maximum, average, and minimum temperatures (°C) of a) golf course, b) northwest, northeast, and square, and c) southwest and southeast rearing ponds used for post-release assessment of westslope cutthroat trout from different hatchery rearing treatments.

Table 14: Mean water chemistry characteristics over 60 days of rearing ponds used in an assessment of the effects of hatchery rearing treatments (CON: conventional, INT: enriched-intermediate, and ENR: enriched-high) on outplant survival and performance of westslope cutthroat trout.

Pond	Treatment	Temperature (°C)	Avg. Food Availability (mg/L)	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Nitrates (mg/L)	pH
Northwest	CON	11.0	0.145	175.09	8.72	0.53	8.64
Northeast	ENR	11.0	0.184	176.44	9.26	0.42	8.54
Southwest	ENR	10.6	0.206	145.14	9.25	0.40	8.36
Southeast	INT	10.6	0.198	148.38	9.28	0.36	8.55
Square	CON	11.0	0.187	151.75	8.39	0.23	8.42
Golf Course	INT	12.9*	0.124	235.29*	9.98*	0.32	8.10

*Indicates significant difference ($p < 0.05$).

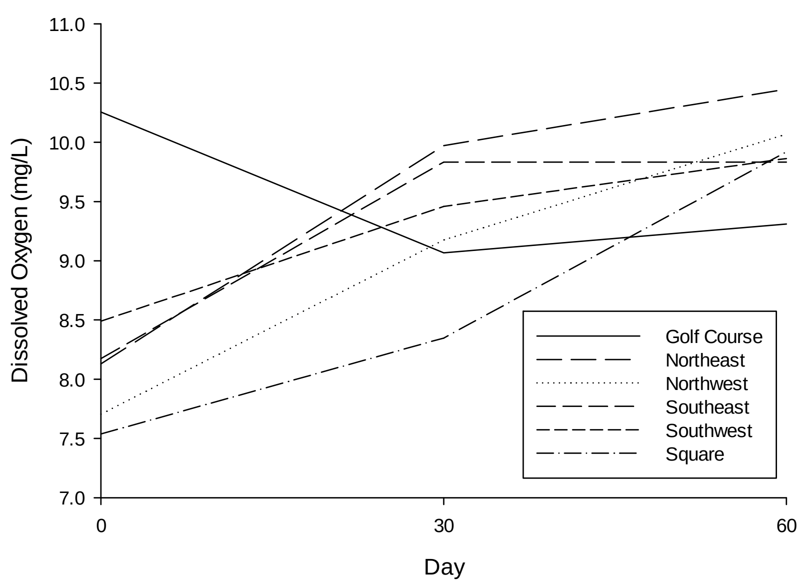


Figure 19: Mean dissolved oxygen content (mg/L) of rearing ponds during 60 day outplanting trial.

Food item density did not differ significantly among the ponds. In all but two of the ponds, food availability increased from day 0 to 30, and decreased from day 30 to 60

(Figure 20). Food availability in the golf course pond was similar at each sampling period, and tended to be lower than the other rearing ponds. By mass, the relative food availability in the ponds was approximately 75% macroinvertebrates and 25% zooplankton. Macroinvertebrates available included ephermeroptera, trichoptera, and diptera and zooplankton consisted primarily of daphniidae. In the square pond, mollusca (freshwater snails) were also prevalent.

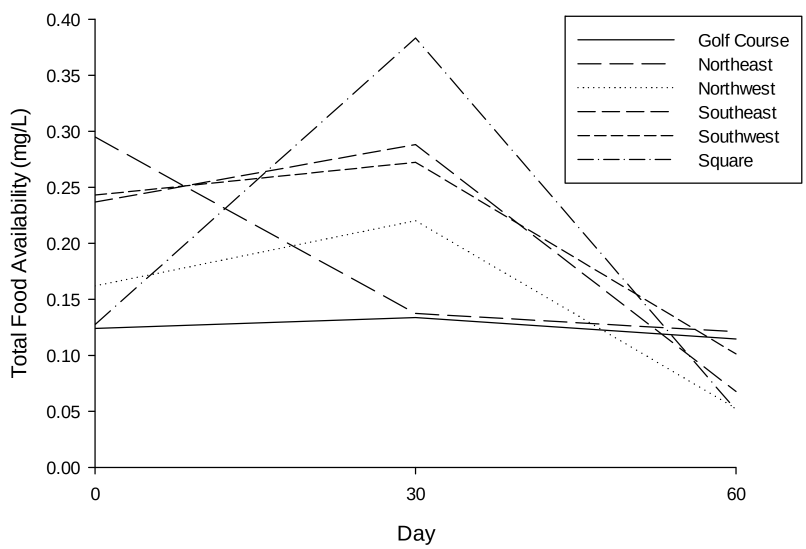


Figure 20: Total food item densities (mg/L) from each rearing pond during a 60 day outplant period.

Apparent fish movement across the barriers in the southeast (INT) and southwest (ENR) ponds occurred, as evidenced by an increase of nine more fish recovered than planted in one pond compared to substantial decreases in numbers in all other ponds, and a very low percentage fish recovered in the adjoining pond (Table 15). As a result of fish movement, these samples were removed from the outplant analyses which limited the sample size of the outplant assessments. Final sample size was one for the INT and ENR treatments and two for the CON treatment, which limited statistical analyses.

Table 15: Number of fish introduced and removed and survival rate of each of the rearing ponds.

Pond	Treatment	# of Fish Introduced	# of Fish Removed	Survival Rate (%)
Northwest	CON	390	319	81.8
Northeast	ENR	234	124	60.0
Southwest	ENR	312	321	102.9[(2)]
Southeast	INT	187	57	30.5
Square	CON	330	115	34.8
Golf Course	INT	1532	638[(1)]	41.6

[(1)] Estimate based on two-pass depletion estimate
[(2)] More fish removed from pond than introduced, evidence of fish movement

Table 16 provides a summary of the data attained at the end of the pond rearing period. Statistical tests could not be applied to the data because of a lack of replication. Survival to the end of the pond rearing period was estimated to be 60.3% in the CON treatment, 41.6% in the INT treatment, and 53.0% in the ENR treatment. Final length and weight varied from 65.5 mm and 3.4 g to 78.2 mm and 5.3 g. The percent increase in length and weight from the time of outplanting to the end of the pond rearing period varied from 17.4% and 80.6% to 52.7% and 265.6%. Dorsal fin index at the end of the pond rearing period was 12.1 in the CON treatment, 12.4 in the INT treatment, and 12.8 in the ENR treatment. Lipid content varied from 2.3 to 4.8%. The percent loss of lipid content from when fish were initially outplanted to when they were removed from the ponds varied from 21.6 to 66.4%.

Table 16: Estimates of response variables measured at the end of a two month pond-rearing period among fish from conventional (CON), enriched-intermediate (INT), and enriched-high (ENR) hatchery rearing environments.

Variable	CON	INT	ENR
Survival (%)	60.3	41.6	53
Length (mm)	78.1	65.5	78.2
Weight (g)	5.3	3.4	5.3
Percent Increase in Length	40.6	17.4	52.7
Percent Increase in Weight	192.9	80.6	256.6
Dorsal Fin Index	12.1	12.4	12.8
Lipid Content (%)	4.2	2.3	4.8
Percent Loss of Lipid Content	37.2	66.4	21.6

DISCUSSION

In this study, the effects of varying levels of hatchery rearing environment complexity on the performance of two species of trout were assessed. Consistent differences occurred in behavior and morphology between the conventional and enriched hatchery rearing environments, with the fish reared in the enriched treatment more closely resembling the traits exhibited by wild fish, as identified from the literature. The two species responded similarly to the rearing treatments in most aspects, but the magnitude of the responses differed. The findings from this study support my hypothesis that enriched hatchery rearing strategies can reduce hatchery deficits common in hatchery fish in both domesticated and wild stocks, however I was unable to determine if the enriched hatchery treatments led to improved outplant survival and performance.

Rearing environment had little apparent effect on survival of cutthroat trout or rainbow trout. Across all treatments, survival over the 60 day rearing trials exceeded 90% in all but one tank. Survival was also near 90% in all treatments during hatchery rearing of cutthroat trout juveniles at Sekokini Springs. Typical trout hatchery survival rates from swim-up to release range from 85-90% (MacKinlay et al. 2004), providing evidence that survival in all treatments was at or better than expected for hatchery salmonids. The high survival rates I observed suggest that incorporation of more complex hatchery rearing environments did not adversely affect survival and hatchery production. Other similar studies evaluating the effects of tank complexity also did not detect significant differences in hatchery survival between conventional and enriched rearing environments (Berejikian et al. 1996, 2000; Fast et al. 2008; Brockmark et al. 2010).

Growth in the hatchery also differed little among the rearing treatments for both species. In westslope cutthroat trout, growth differed between the ENR and CON environments, although the observed differences of 5 mm and 0.5 g in length and weight are likely not biologically significant. Recent work by Brockmark and Johnsson (2010) also found no differences in growth among various levels of structure and density in hatchery rearing environments using brown trout. Similarly, Berejikian et al. (2000) found no difference in percent daily growth rate among steelhead reared in conventional and enriched (overhead and submerged cover) tanks. Therefore, use of more enriched or 'natural' rearing environments does not appear to adversely affect growth in hatchery reared salmonids. The lack of growth differences among cutthroat trout and rainbow trout in my study support the hypothesis that fish will respond similarly to alterations in the hatchery environment regardless of domestication level. During hatchery rearing at Sekokini, growth among westslope cutthroat trout was lower in the ENR treatment, although this was likely a result of tank design as clogging of the underwater feed pipe was common and the underwater feed system might not have been efficient at distributing the feed throughout the length of the large tanks used in the Sekokini hatchery trials. The clogging and delivery issues with the submerged feed delivery system point to the need for further testing of suitable underwater food delivery systems.

Cover seeking behavior and fright response were influenced by rearing treatment in both species. Fish from the INT and ENR treatments were much more likely to use cover than fish from the CON treatment. My findings support those of Roadhouse et al. (1986), who found that lake trout *Salvelinus namaycush* reared with overhead cover exhibited increased startle behavior. However, a similar study by Berejikian et al. (2000) suggested that the innate preference for cover in juvenile steelhead was not influenced by cover availability in the hatchery. Berejikian et al. (2000) did differ slightly from my study, as their measure of cover use was performed in quasi-natural stream sections, while my measurements of cover use and fright response were performed in a controlled laboratory setting. My results do corroborate the prediction of Wiley et al. (1993b), who suggested that hatchery fish could be trained to use cover by making it available in the hatchery environment. Wiley et al. (1993b) further suggested that fish trained to use cover would survive better in the wild as the ability to use cover effectively could improve predator avoidance and foraging efficiency.

Agonistic behavior varied greatly among the rearing treatments and between the species. I found that fish reared under conventional hatchery methods were more aggressive than those from enriched rearing treatments and rainbow trout exhibited higher aggression levels than did cutthroat trout. Findings from several studies have noted that salmonids reared under conventional hatchery rearing methods are more aggressive than their wild counterparts (e.g., Fenderson et al. 1968; Swain and Ridell 1990; Deverill et al. 1999). However, my results showed that the high levels of aggression common in conventionally raised fish were reduced by rearing fish in more complex environments, and that a similar reduction in aggression could be induced in both highly domesticated rainbow trout and a wild strain of westslope cutthroat trout.

My results indicated that fish reared under overhead cover and remote (belt) feeding and with overhead and submerged cover and submerged feeding, had similar levels of aggression. Aggression assessments on complex hatchery rearing environments are mixed, some studies have found no aggression differences (e.g., Berejikian et al. 2000; Riley et al. 2005), whereas others have found reductions in aggression induced by rearing environment complexity similar to my study. Mesick (1988) found that structural complexity reduced ‘intruder pressure’ for salmonids, thus causing individual fish to experience reduced levels of aggression. Berejikian and Tezak (2005) demonstrated that higher tank complexity was associated with reduced aggression, indicated by less nipping-induced fin damage in juvenile steelhead. Structural complexity has also been shown to reduce aggression in other species, exhibited by the work of Basquill and Grant (1998) on zebra fish *Danio rerio*. I also found that CON fish expressed their aggression differently than fish from the enriched treatments, exhibiting more nips than threats and displays. The reduction in physical aggression may be associated with greater energetic efficiency (Bachman 1984; Deverill et al. 1999) and development of social hierarchy once fish are outplanted. My results suggest that modifying the hatchery rearing environment to encourage development of a social hierarchy and mimicking more natural feeding conditions can substantially reduce the high levels of aggression common among hatchery trout.

The aggression findings held for both species tested, although the difference in aggression between the species might not be attributable solely to hatchery rearing environment. The strain of rainbow trout used was highly domesticated and has long been artificially selected for traits beneficial to hatchery production. One of the artificial traits common among hatchery fish is aggression, as more aggressive fish likely experience higher growth rates in the hatchery (Huntingford 2004). It is also known that rainbow trout are generally more aggressive than cutthroat trout, as rainbow trout were much more aggressive than cutthroat trout in matched pair behavior assessments (Nilsson and Northcote 1981). It is most likely a combination of these factors that explain the higher aggression levels seen in rainbow trout in this study. These results suggest that the aggression typical of hatchery fish, regardless of domestication level, can be reduced to levels that may improve their performance in natural conditions and reduce the competitive advantage over their wild counterparts, often cited as a major problem with hatchery supplementation programs (Weber and Fausch 2003).

Predator avoidance trials with rainbow trout yielded inconclusive results. Fish reared in the ENR treatment did have the lowest odds of being consumed by a predator, but high variation among the trials, generally low predation levels, and inadequate replicates limited the analysis. Also, covariates such as predator hunger and aggression were not quantified, leaving the possibility for unexplained variation among the findings. The findings from the cover seeking behavior trials combined with the results of the predator trials might imply that fish reared under ENR conditions have the potential to better avoid predators once outplanted. It is believed that predation of juveniles is one of the most important factors limiting survival of hatchery outplants (Healey 1982; Johnsson and Abrahams 1991), because of this predator training has become a common fish culture technique to improve survival of hatchery fish. For example, Berejikian et al. (1999)

found that hatchery fish can be trained to avoid predators in a controlled hatchery setting. This and other similar studies (e.g., Olla and Davis 1988; Hawkins et al. 2007) mostly rely on predator exposure to train hatchery fish. The results of this study testing if rearing environment alone can predispose better predator avoidance is unique and provides moderate evidence that it can.

Rearing fish under enriched conditions greatly improved average fin condition. Among both species, the ENR treatment had substantially better fin condition than the CON treatment. Multiple explanations for the poor fin condition typical of hatchery fish have been proposed including fin nipping (Abbott and Dill 1985; Bosakowski and Wagner 1994), high rearing density (Soderberg and Meade 1987), diet (Lellis and Barrows 1997), and tank surface material (Abbot and Dill 1985; Bosakowski and Wagner 1994). Poor fin condition has long been noted as an issue with hatchery-reared fish (e.g., Agersborg 1933; Wolf 1938; Butcher 1947) and is considered an indicator of overall fish health (Schneider and Nicholson 1980; Wagner et al. 1997; Latremouille 2003). Similar to my findings, a 45-55% decrease was found in the dorsal fin height of juvenile steelhead from a conventional rearing environment compared to an enriched (overhead and submerged cover) hatchery rearing environment (Berejikian and Tezak 2005). The authors suggested that the improved dorsal fin heights found in their study was from lower aggression levels in tanks with structure. Although I made no attempts to determine the cause of the dorsal fin height variation in this study, the same reasoning might explain why dorsal fin height was so different between the ENR and CON treatments. Among cutthroat trout, the dorsal fin index of the INT treatment was more similar to the CON treatment than the ENR. While the experimental aggression levels between the INT and ENR treatments were similar, observations of the feeding aggression between the treatments were quite different. Fish from the ENR treatment tended to remain in feeding lanes, waiting for feed to flow near them. Fish from the INT treatment tended to aggregate near the belt feeder and develop feeding swarms when food was delivered. This difference in feeding aggression might account for the lower fin index among the INT fish. Additionally, the intruder pressure hypothesis proposed by Mesick (1988) might explain the difference in dorsal fin index between the INT and ENR, as the number of encounters between fish might have been substantially lower in the ENR treatment because of the submerged cover, which would have reduced the number of bouts between fish in the rearing tank. Contributing the differences in dorsal fin index to aggression is one hypothesis, however, during the territory trials very few of the nips I observed were directed at the dorsal fin. Other proposed hypotheses mentioned above can be excluded in this study, as fish were fed the same diet and reared in the same aluminum or fiberglass tanks. The other common explanation for fin erosion in hatchery fish is crowding stress (Soderberg and Meade 1987). The rearing densities of the hatchery tanks were similar. However, the distributions of the fish in the tanks differed greatly. As mentioned earlier, fish from the CON and INT treatments tended to remain aggregated, while fish from the ENR treatment were evenly distributed throughout the tank. This would have effectively increased the densities experienced in the CON and INT treatments as they used less of the available space in the rearing tank, possibly accounting for the lower dorsal fin indexes. It should also be noted that the reduction in dorsal fin index found in the CON treatment during hatchery rearing was not apparent at the end of the outplanting phase, suggesting that the deficit is overcome after time in a natural environment.

Complexity in the rearing environment was associated with improved coloration for both cutthroat and rainbow trout. The improved coloration found in ENR environments was likely an artifact of the color variability present in the rearing environments. Coloration in fish is largely a function of the surrounding environment (Fujii 1969). The CON treatment was a barren silver tank with no shading variability, while the ENR treatment had overhead shading and submerged cover which increased the variability and availability of shading in the rearing tank. Relating the shading variation and availability present in the rearing treatments to Fujii's description of coloration in fish explains the differences found among the rearing treatments in this study. Differences in coloration have also been noted in NATURES studies, as enriched environments (overhead and submerged cover) produced more cryptic coloration than conventional environments in Pacific salmon species (Maynard et al. 2003). Improving the coloration of hatchery fish could be beneficial to predator avoidance (Donnelly and Dill 1984) and social interactions (Berejikian et al. 1999) in the wild. Further improvements could be made to enriched hatchery rearing environments that may enhance the coloration of hatchery-reared fish, such as using natural substrate, intermittent overhead cover, and more natural tank coloration (Donnelly and Whoriskey 1991; Maynard et al. 1995).

The response to a stressor (i.e., cortisol, glucose, and lactate levels) showed no significant trends among the rearing treatments. It might be anticipated that fish from an enriched hatchery rearing environment would show greater response to stressors as they are reared under more natural settings, whereas fish from conventional environments become acclimated to stressors such as human presence, high levels of aggression, and feeding competition. This assumption would suggest that fish from enriched hatchery environments might have a more sensitive fight or flight response in the wild, thus making them more likely to perform better than fish from a conventional environment; however I found no evidence to support this hypothesis.

Hatchery rearing environment had no apparent effect on lipid or protein content. Overall, rainbow trout had slightly higher lipid content than cutthroat trout. I have found no other studies directly measuring body composition among hatchery reared fish from altered hatchery environments. The difference in lipid content between the species might be explained by the domestication differences between the stocks tested. Domesticated brook trout *Salvelinus fontinalis* had higher fat content than fish from wild stock, suggesting that more domesticated stocks contain higher levels of lipid content (Vincent 1960). The increase of lipid content in rainbow trout suggests that the more domesticated rainbow trout was better suited to put on lipid content in the hatchery setting than the less domesticated westslope cutthroat trout.

Combinations of the measured response variables were able to clearly discriminate between the rearing treatments. For cutthroat trout, the grouping of length, dorsal fin index, cover use, and lipid content distinguished the rearing treatments from each other, as did dorsal fin index, lipid content, and condition for rainbow trout. Other variables were significantly different among the treatments, but these combinations were most significant to the discriminant function analysis. All rearing treatment comparisons were statistically different in the discriminant function space, indicating that fish from each rearing treatment were unique. These findings and the high reclassification percentages indicate that the combination of all of the response variables, not just the univariate analyses, clearly differentiate fish from each of the rearing treatments.

The hatchery rearing results from Sekokini Springs corroborated most of the findings from the rearing trials performed at BFTC. The survival and fin condition results suggest that the treatment effects I found are applicable to multiple species, locations, and scales of hatchery production. The decrease in growth that occurred within the Sekokini hatchery can mostly be explained by lower water temperatures during the first month of rearing and the problems experienced with the submerged feed system (i.e., poor feed distribution and clogging).

The results from the outplant survival data were inconclusive because of known fish movement among two of the ponds, reducing the number of total number of samples from 6 to 4. I was unable to detect any conclusive trends in survival among the rearing treatments. However, Maynard et al. (1995) found a 50% increase in post-release survival of juvenile Chinook salmon from semi-natural (substrate, overhead and submerged cover) hatchery rearing environments compared to conventionally reared fish. Smolt-to-adult survival of coho salmon *Oncorhynchus kisutch* from seminatural ponds (rock bottoms and woody debris) was higher than those from control ponds (dirt bottom and barren) in 2 years out of a 3-year study (Fuss and Byrne 2002). More recently, Maynard et al. (2003) summarized numerous NATURES studies which have tested the effects of semi-natural rearing environments on anadromous salmonid survival, finding mixed results. Over a 4-year study, Maynard reports significant differences in Chinook salmon smolt survival in 3 of 11 trials, indicating the inconsistent nature of the survival results. Not all enriched hatchery treatments have been found to improve post-release survival. Juvenile and adult Chinook salmon reared in semi-natural (camouflage raceway, surface and underwater structure, underwater feeder) and optimum conventional (concrete raceway, surface feeding) hatchery rearing treatments provided insufficient evidence of survival differences (Fast et al. 2008). Additionally, Tatara et al. (2009) found no difference in survival among conventional and enriched (overhead and submerged cover, underwater food delivery) hatchery-reared steelhead over a 6-week period in stream sections. However, more recent results from Brockmark et al. (2010) have found that the density at which hatchery fish are reared can greatly influence their survival in the wild, suggesting that further enriched hatchery strategies in combination with increased tank complexity might provide higher post-release survival of hatchery fish.

I was unable to test for differences in growth among the rearing treatments during the pond-rearing period, although the largest percentage increases in length and weight occurred in the ENR treatment. The current understanding of growth among outplanted enriched hatchery-reared salmonids is inconsistent. Tatara et al (2009) found no difference in growth rates among conventional and enriched hatchery-reared steelhead, but Berejikian et al. (2000) found that fish reared in enriched hatchery conditions exhibited greater increases in length than did conventionally reared fish in a quasi-natural stream when fish from the treatments were intermixed. Fish from the ENR treatment also lost about half as much lipid content as the other treatments during the pond-rearing period. It has long been known that lipid content is linked to over-winter survival (Reimers 1963). A recent study by Biro et al. (2004) has estimated a minimal lipid threshold for overwinter survival in rainbow trout to be approximately 0.9% lipid content at the end of winter. The Biro et al. study also found that fish lost 60-80% of their lipid content during an overwinter period of 5 months. Assuming these values are similar for the closely related westslope cutthroat trout, the lipid values of fish from the CON and INT treatments would be below or near the minimal lipid threshold after overwintering, while the ENR treatment would remain slightly above the threshold. These findings may have significant implications on the potential over-winter survival of outplanted hatchery fish. Interpretation of the growth analyses during the outplant period should bear in mind that the treatments were stocked into different rearing ponds, where temperature, water chemistry, and food availability were unique.

Due to space limitations, I was unable to determine if the improvements in behavior and morphology among fish from the ENR treatment was due to structure, feed delivery method, or the combination of the two. Future work should attempt to resolve which suit of hatchery alterations induces the desired changes in behavior and morphology. Interpretation of the results of my study are limited by the lack of outplant survival and performance results. This has been an issue with many hatchery rearing environment studies and unfortunately my attempt to evaluate outplant survival and performance was unsuccessful. Any inferences made toward outplant success are speculative at best. Another limitation of this study is the lack of direct comparison of behavior, morphology, and physiology between hatchery reared and wild fish. Based on the literature, I assumed that the characteristics of the enriched rearing treatment were more similar to wild fish. This assumption lacks direct testing in my study and the conclusions drawn should bear that in mind. Further research examining additional rearing alterations, the long term effects of hatchery rearing environment (i.e. long term survival, reproductive fitness, habitat use, and diet), and comparing the effectiveness of hatchery outplants versus RSI's would improve our understanding of the conservation hatchery strategies evaluated here.

Conclusions and Management Implications

The findings of this study have shown that reductions in the common differences between hatchery fish and their wild counterparts can be attained with simple alterations to the hatchery rearing environment. It is believed that the differences induced by the enriched hatchery environments might lead to improved survival in the wild, however I was unable to determine outplant survival differences in this study. My findings also suggest that the role of early learning greatly influences the behavior of hatchery reared salmonids and that native fish recovery efforts using captive rearing will likely benefit from incorporating learning and training into the hatchery environment. The results from this study, combined with Berejikian et al. (1999), Strand et al. (2010), and many others provide convincing evidence that the composition of the hatchery environment significantly influences the behaviors of hatchery-reared fish. In this study, I found the composition of the hatchery rearing environment during this critical early learning phase of development likely plays a more significant role in the behavior and performance of hatchery-reared fish than species or level of domestication. A common viewpoint in hatchery research is that maintaining wild genetics among hatchery fish preserves natural behaviors (Swain and Riddell 1990). The findings of this study demonstrate that the changes in behavior and morphology among inland salmonids occurred primarily because of the hatchery rearing environment, whereas species and domestication (genetic) differences appeared to have a much smaller influence.

The current use of hatchery-reared fish for the conservation or reintroduction of native species is becoming increasingly prevalent. This requires managers to evaluate their hatchery procedures to get the most effective use of the limited genetically pure stocks and personnel resources to achieve native species conservation goals. I have found that minimal alterations to the rearing environment can reduce behavioral and morphological differences between hatchery fish and wild fish. The alterations to the hatchery environment did not decrease production, increase cleaning time, or negatively affect water quality. Although I was not able to document clear differences in post-release survival, findings from behavioral, morphological, and physiological assessments and post-release growth differences provide suggestive evidence that the effectiveness of native species conservation programs utilizing hatchery-reared fish would benefit from enriched hatchery rearing strategies. Managers should also be aware of the significant role that learning, training, and imprinting have on captive reared animals. Variable environments and training have long been used for endangered birds and mammals, producing more elastic behaviors and effective conservation efforts (Strand et al. 2010). Applying this understanding to fish species is a burgeoning area of research and is crucial to the future of conservation hatchery programs. My study suggests that the early rearing environment plays an important role in the development and expression of ‘wild’ behaviors among hatchery reared salmonids. Maintaining a wild gene pool is not enough. For hatchery stocks to perform best in the natural environment, learning and training in the hatchery environment is critical.

REFERENCES CITED

Abbot, J. C., and L. M. Dill. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 42: 1702-1706.

Agersborg, H. P. K. 1933. Salient problems in the artificial rearing of salmonid fishes, with special reference to intestinal fungisitosis and the cause of white-spot disease. Transactions of the American Fisheries Society 63:240-250.

Allen, S. E., H. M. Grimshaw, J. A. Parkinson, and C. Quarmby. 1974. Chemical analysis of ecological materials. Blackwell, Oxford, England.

Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Transactions of the American Fisheries Society 109:537-543.

Anderson, D. P. 1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. American Fisheries Society Symposium 8:38-50.

Anderson, R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Anonymous. 2003. Hatchery research center at Fall Creek: concept document. Oregon State University and Oregon Department of Fish and Wildlife, Hatchery Research Center. Available: http://www.dfw.state.or.us/OHRC/materials/cp.pdf (February 2009).

AOCS (American Oil Chemists' Society). 2009a. Rapid determination of oil/fat utilizing high-temperature solvent extraction. AOCS standard procedure Am 5-04 *in* D. Firestone, editor. Official methods and recommended practices of the AOCS, 6th edition. AOCS press, Champaign, Illinois.

AOCS (American Oil Chemists' Society). 2009b. Generic combustion method for determination of crude protein. AOCS official method Ba 4e-93 *in* D. Firestone, editor. Official methods and recommended practices of the AOCS, 6th edition. AOCS press, Champaign, Illinois.

Bachman, R. A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Transactions of the American Fisheries Society 113:1-32.

Basquill, S. P. and J. W. A. Grant. 1998. An increase in habitat complexity reduces aggression and monopolization of food by zebra fish (*Danio rerio*). Canadian Journal of Zoology 76:770-772.

Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Canadian Journal of Fisheries and Aquatic Sciences 52:2476-2482.

Berejikian, B. A., S. B. Mathews, and T. P. Quinn. 1996. Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. Canadian Journal of Fisheries and Aquatic Sciences 53:2004-2014.

Berejikian, B. A., R. J. F. Smith, E. P. Tezak, S. L. Schroder, and C. M. Knudsen. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Canadian Journal of Fisheries and Aquatic Sciences 56:830-838.

Berejikian, B. A., E. P. Tezak, T. A. Flagg, A. L. LaRae, E. Kummerow, and C. V. W. Mahnken. 2000. Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. Canadian Journal of Fisheries and Aquatic Sciences 57:628-636.

Berejikian, B. A., E. P. Tezak, S.C. Riley, and A.L. LaRae. 2001. Competitive ability and social behavior of juvenile steelhead reared in enriched and conventional hatchery tanks and a stream environment. Journal of Fish Biology 59:1600-1613.

Berejikian, B. A., and E. P. Tezak. 2005. Rearing in enriched hatchery tanks improves dorsal fin quality in juvenile steelhead. North American Journal of Aquaculture 67:289-293.

Biro, P. A., A. E. Morton, J. R. Post, and E. A. Parkinson. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 61:1513-1519.

Bosakowski, B. and E. J. Wagner. 1994. 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.

Brockmark, S., and J. I. Johnsson. 2010. Reduced hatchery rearing density increases social dominance, postrelease growth, and survival in brown trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences 67: 288-295.

Brockmark, S., B. Adriaenssens, and J. I. Johnsson. 2010. Less is more: density influences the development of behavioral life skills in trout. Proceeding of the Royal Society B 277: 3035-3043.

Brown, C. and K. Laland. 2001. Social learning and life skills training for hatchery reared fish. Journal of Fish Biology 59:471-493.

Brown, C. and R. L. Day. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish and Fisheries 3:79-94.

Butcher, A. D. 1947. Ichthyophthiriasis in Australian trout hatchery. The Progressive Fish-Culturist 9: 21-26.

Craig, J. F., M. J. Kenley, and J. F. Talling. 1978. Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis. Freshwater Biology 8: 525-590.

Deverill, J. I., C. E. Adams, and C. W. Bean. 1999. Prior residence, aggression and territory acquisition in hatchery-reared and wild brown trout. Journal of Fish Biology 55:868-875.

Donnelly, W. A., and F. G. Whoriskey Jr. 1991. Background-color acclimation of brook trout for crypsis reduces risk of predation by Hooded Mergansers *Lophodytes cucullatus*. North American Journal of Fisheries Management 11:206-211.

Einum, S., and I. A. Fleming. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. Journal of Fish Biology 50:634-651.

Einum, S., and I. A. Fleming. 2001. Implications of stocking: ecological interactions between wild and released salmonids. Nordic Journal of Freshwater Research 75:56-70.

Fast, D. E., D. Neeley, D. T. Lind, M. V. Johnston, C. R. Storm, W. J. Bosch, C. M. Knudsen, S. L. Schroder, and B. D. Watson. 2008. Survival comparison of spring Chinook salmon reared in a production hatchery under optimum conventional and seminatural conditions. Transactions of the American Fisheries Society 137:1507-1518.

Fenderson, O. C., W. H. Everhart, and K. M. Muth. 1968. Comparative agonistic and feeding behavior of hatchery-reared and wild salmon in aquaria. Journal of the Fisheries Research Board of Canada 25:1-14.

Ford M. J., and J. J. Hard. 2000. Does traditional hatchery production help conserve wild salmon – a comment on the Fall Creek coho hatchery controversy. Northwest Fisheries Science Center, Unpublished manuscript. Available: www.nwfsc.noaa.gov/cbd/LannanResponse.pdf. (February 2009).

Foster, L. B. and R. T. Dunn. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clinical Chemistry 20: 365-368.

Fujii, R. 1969. Chromatophores and Pigments. Pages 307 – 353 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology volume III: reproduction and growth; bioluminescence, pigments, and poisons, Academic Press, London.

Fuss, H. and J. Byrne. 2002. Differences in survival and physiology between coho salmon reared in seminatural and conventional ponds. North American Journal of Aquaculture 64: 267-277.

Grisak, G., and B. Marotz. 2003. South Fork Flathead watershed westslope cutthroat trout conservation program. Bonneville Power Administration Report DOE/BP-00005043-1, Project 1991-01903, Portland, Oregon.

Gross, M. R. 1998. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. Canadian Journal of Fisheries and Aquatic Sciences 55:131-144.

Hawkins, L. A. 2007. A test of how predator conditioning influences survival of hatchery-reared Atlantic salmon, *Salmo salar*, in restocking programmes. Fisheries Management and Ecology 14:291-293.

Healey, M. C. 1982. Timing and relative intensity of size selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Canadian Journal of Fisheries and Aquatic Sciences 39:952-957.

Hesthagen, T., and B. O. Johnsen. 1989. Lake survival of hatchery and pre-stocked pond brown trout, *Salmo trutta* L. Aquaculture Research 20:91-95.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries 17(1):5-8.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic-effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48:945-957.

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

Johnsen, B. O., and O. Ugedal. 1989. Feeding by hatchery-reared brown trout, *Salmo trutta* L. released in lakes. Aquaculture and Fisheries Management 20:97-104.

Johnsson, J. I., and M. V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Canadian Journal of Fisheries and Aquatic Sciences 48:243-247.

Johnsson, J. I., H. Johan, and I. A. Fleming. 2001. Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. Canadian Journal of Fisheries and Aquatic Sciences 58:788-794.

Kaeding, L. R., and G. D. Boltz. 2004. Use of remote-site incubators to produce arctic grayling fry of wild parentage. North American Journal of Fisheries Management 24:1031-1037.

Keenleyside, M. H. A. and F. T. Yamamoto. 1962. Territorial behaviour of juvenile Atlantic salmon (Salmo salar L.). Behaviour 19: 139-169.

Kindschi, G. A. 1987. Method for quantifying degree of fin erosion. The Progressive Fish-Culturist 49:314-315.

Kostow, K. E. 2004. Differences in juvenile phenotypes and survival between hatchery stocks and a natural population provide evidence for modified selection due to captive breeding. Canadian Journal of Fisheries and Aquatic Sciences 61:577-589.

Kostow, K. E. 2009. Factors that contribute to the ecological risks of salmon and steelhead hatchery programs and some mitigating strategies. Reviews in Fish Biology and Fisheries 19:9-31.

Latremouille, D. N. 2003. Fin erosion in aquaculture and natural environments. Reviews in Fisheries Science 11:315-335.

Lee, J. S., and B. A. Berejikian. 2008. Effects of the rearing environment on average behavior and behavioral variation in steelhead. Journal of Fish Biology 72:1736-1749.

Lellis, W. A., and F. T. Barrows. 1997. The effect of diet on dorsal fin erosion in steelhead trout (*Oncorhynchus mykiss*). Aquaculture 156: 229-240.

Liknes, G. A. and P. J. Graham. 1988. Westslope cutthroat trout in Montana: life history, status, and management. American Fisheries Society Symposium 4:53-60.

MacKinlay, D. D., S. Lehmann, J. Bateman, and R. Cook. 2004. Pacific salmon hatcheries in British Columbia. American Fisheries Society Symposium 44:57-75.

Maynard, D. J., T. A. Flagg, and C. V. W. Mahnken. 1995. A review of seminatural culture strategies for enhancing postrelease survival of anadromous salmonids. American Fisheries Society Symposium 15:307-314.

Maynard, D. J., S. Riley, B. A. Berejikian, and T. A. Flagg. 2003. Development of a natural rearing system to improve supplemental fish quality. Bonneville Power Administration Report DOE/BP-00000548-1, Project 1991-05500, Portland, Oregon.

McDonald, D. G., C. L. Milligan, W. J. McFarlane, S. Croke, S. Currie, B. Hooke, R. B. Angus, B. L. Tufts, and K. Davidson. 1998. Condition and performance of juvenile Atlantic salmon (*Salmo salar*): effects of rearing practices on hatchery fish and comparison with wild fish. Canadian Journal of Fisheries and Aquatic Sciences 55:1208-1219.

McMullin, S. L., and T. Dotson. 1988. Use of McBride Lake strain Yellowstone cutthroat trout for lake and reservoir management in Montana. American Fisheries Society Symposium 4:42-44.

Mesa, M. G. 1991. Variation in feeding, aggression, and position choice between hatchery and wild cutthroat trout in an artificial stream. Transactions of the American Fisheries Society 120:723-727.

Mesick, C. F. 1988. Effects of food and cover on numbers of apache and brown trout establishing residency in artificial stream channels. Transactions of the American Fisheries Society 117:421-431.

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

Mobrand, L. E., J. Barr, L. Blankenship, D. E. Campton, T. T. P. Evelyn, T. A. Flagg, C. V. W. Mahnken, L. W. Seeb, P. R. Seidel, and W. W. Smoker. 2005. Hatchery reform in Washington state: principles and emerging issues. Fisheries 30(6):11-22.

(MFWP) Montana Fish, Wildlife, and Parks. 2004. Hatchery and genetic management plan resident fish edition (HGMP-RF). Kalispell, Montana.

(MFWP) Montana Fish, Wildlife, and Parks. 2006. South Fork Flathead watershed westslope cutthroat trout conservation program. Kalispell, Montana.

(MFWP) Montana Fish, Wildlife, and Parks. 2007. Memorandum of understanding and conservation agreement for westslope cutthroat trout and Yellowstone cutthroat trout. Helena, Montana.

Moyle, P. B. 1969. Comparative behavior of young brook trout of domestic and wild origin. The Progressive Fish-Culturist 31:51-59.

Newman, M. A. 1956. Social behavior and interspecific competition in two trout species. Physiological Zoology 29:64-81.

Nilsson, N. A. and T. G. Northcote. 1981. Rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*) interactions in coastal British Columbia lakes. Canadian Journal of Fisheries and Aquatic Sciences 38: 1228-1246.

Olla, B. L., M. W. Davis, and C. H. Ryer. 1998. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. Bulletin of Marine Science 62:531-550.

Pickering, A. D., R. Griffiths, and T. G. Pottinger. 1987. A comparison of the effects of overhead cover on the growth, survival, and haematology of juvenile Atlantic salmon, *Salmo salar*L., brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. Aquaculture 66:109-124.

Ramsey, F. L. and D. W. Schafer. 2002. The statistical sleuth: a course in methods of data analysis, 2nd edition. Duxbury, Pacific Grove, California.

Redding, J. M., C. B. Schreck, E. K. Birks, and R. D. Ewing. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology 56: 146-155.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 1-382.

Riley, S. C., C. P. Tatara, and J. A. Scheurer. 2005. Aggression and feeding of hatchery-reared and naturally reared steelhead (*Oncorhynchus mykiss*) fry in a laboratory flume and a comparison with observations in natural streams. Canadian Journal of Fisheries and Aquatic Sciences 62:1400-1409.

Roadhouse, S., M. J. Saari, D. Roadhouse, and B. A. Pappas. 1986. Behavioral and biochemical correlates of hatchery rearing methods on lake trout. The Progressive Fish-Culturist 48:38-42.

Schneider, R. and B. L. Nicholson. 1980. Bacteria associated with fin rot disease in hatchery-reared Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 37: 1505-1513.

Schreck, C. B. 2000. Accumulation and long-term effects of stress in fish. Pages 147-158 *in* G. P. Moberg and J. A. Mench, editors. The biology of animal stress. CABI Publishing, New York.

Shepard, B. B., B. E. May, and W. Urie. 2003. Status of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) in the United States: 2002. WCT Multi-state Assessment.

Soderberg, R. W. and J. W. Meade. 1987. Effects of rearing density on growth, survival, and fin condition of Atlantic salmon. The Progressive Fish-Culturist 49: 280-283.

Strand, D. A., A. C. Utne-Palm, P. J. Jakobsen, V. A. Braithwaite, K. H. Jensen, and A. G. V. Salvanes. 2010. Enrichment promotes learning in fish. Marine Ecology Progress Series 412: 273-282.

Swain, D. P. and B. E. Riddell. 1990. Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 47: 566-571.

Tatara, C. P., S. C. Riley, and J. A. Scheurer. 2008. Environmental enrichment in steelhead (*Oncorhynchus mykiss*) hatcheries: field evaluation of aggression, foraging, and territoriality in natural and hatchery fry. Canadian Journal of Fisheries and Aquatic Sciences 65:744-753.

Tatara, C. P., S. C. Riley, and J. A. Scheurer. 2009. Growth, survival, and habitat use of naturally reared and hatchery steelhead fry in streams: effects of an enriched hatchery rearing environment. Transactions of the American Fisheries Society 138:441-457.

Turek, J, T. Randák, P Horkỳ, V. Žlábek, J. Velíšek, O. Slavík, and R. Hanák. 2010. Post-release growth and dispersal of pond and hatchery-reared European grayling *Thymallus thymallus* compared with their wild conspecifics in a small stream. Journal of Fish Biology 76: 684-693.

Usher, M. L., C. Talbot, and F. B. Eddy. 1991. Effects of transfer to seawater on growth and feeding in Atlantic salmon smolts (*Salmo salar* L.). Aquaculture 94:309-326.

Van Eimeren, P. 1996. Westslope cutthroat trout *Oncorhynchus clarki lewisi* conservation assessment for inland cutthroat trout: distribution, status, and habitat management implications. US. Department of Agriculture, Forest Service, Intermountain Region, Ogden, Utah. Pages 1-10.

Vilhunen, S. 2006. Repeated antipredator conditioning: a pathway to habituation or to better avoidance?. Journal of Fish Biology 68:25-43.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Transactions of the American Fisheries Society 128:302-316.

Wagner, E. J., T. Jeppsen, R. Arndt, M. D. Routledge, and Q. Bradwisch. 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.

Weber, E. D., and K. D. Fausch. 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. Canadian Journal of Fisheries and Aquatic Sciences 60:1018-1036.

Werner, I., M. R. Viant, E. S. Rosenblum, A. S. Gantner, R. S. Tjeerdema, and M. L. Johnson. 2006. Cellular responses to temperature stress in steelhead trout (*Oncorhynchus mykiss*) parr with different rearing histories. Fish Physiology and Biochemistry 32:261-273.

White, R. J., J. R. Karr, and W. Nehlsen. 1995. Better roles for fish stocking in aquatic resource management. American Fisheries Society Symposium 15:527-547.

Wiley, R. W., R. A. Whaley, J. B. Satake, and M. Fowden. 1993a. Assessment of stocking hatchery trout: a Wyoming perspective. North American Journal of Fisheries Management 13:160-170.

Wiley, R. W., R. A. Whaley, J. B. Satake, and M. Fowden. 1993b. An evaluation of the potential for training trout in hatcheries to increase poststocking survival in streams. North American Journal of Fisheries Management 13:171-177.

Wills, T. C. 2006. Comparative abundance, survival, and growth of one wild and two domestic brown trout strains stocked in Michigan rivers. North American Journal of Fisheries Management 26:535-544.

Wolf, L. E. 1938. Effect of amount of food on fin condition of fingerling trout. The Progressive Fish-Culturist 5: 16-18.

Woodward, C. C. and R. J. Strange. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116:574-579.

APPENDIX A

DISCRIMINANT FUNCTION ANALYSIS DATA

Data used for discriminant function analysis (DFA) for multivariate responses that discriminate the subjects into different groups among westslope cutthroat trout (WCT) and rainbow trout (RBT). Variables include survival, length, weight, condition, dorsal fin index, aggression, cover seeking behavior, lipid content, protein content, and cortisol level.

Species	Treatment	Survival (%)	Length (mm)	Weight (g)	Condition (K)	Dorsal Fin Index	Aggression	Cover	Lipid Content (%)	Protein Content (%)	Cortisol (ng/mL)
CTT	CON	96.6	56.1	1.9	1.09	8.34	185	0.0	7.07	15.33	47.7
CTT	CON	96.7	56.8	2.0	1.09	7.58	205	0.5	7.40	16.00	57.0
CTT	CON	97.0	56.9	2.1	1.12	8.33	345	0.5	7.42	15.47	63.7
CTT	CON	93.8	57.2	2.0	1.07	8.24	138	0.0	7.25	15.50	55.2
CTT	INT	95.6	57.9	2.3	1.16	8.32	74	1.7	6.82	15.61	35.9
CTT	INT	95.1	59.7	3.3	1.55	8.52	111	2.7	6.99	15.08	32.3
CTT	INT	93.8	61.3	2.6	1.12	8.03	118	2.5	7.11	15.77	43.6
CTT	INT	92.3	59.7	2.3	1.10	8.85	124	2.8	7.23	15.78	49.4
CTT	ENR	93.1	59.4	2.3	1.12	10.79	92	0.0	6.76	15.19	60.9
CTT	ENR	78.6	61.3	2.6	1.13	10.78	116	1.8	7.33	15.49	46.6
CTT	ENR	92.8	61.1	2.5	1.09	10.12	88	1.6	7.41	15.10	51.9
CTT	ENR	90.3	60.3	2.4	1.08	11.48	102	3.4	6.97	15.68	48.8
RBT	CON	95.0	63.9	3.1	1.19	8.45	367	1.6	7.43	15.39	29.5
RBT	CON	95.8	58.9	2.3	1.14	7.45	292	0.0	7.97	14.84	49.8
RBT	CON	98.3	58.0	2.3	1.19	8.79	367	0.0	8.35	15.20	44.8
RBT	CON	94.5	54.5	2.0	1.24	8.89	314	0.0	8.20	14.86	37.4
RBT	INT	94.9	63.5	3.0	1.17	10.79	292	0.3	6.84	14.98	89.0
RBT	INT	93.8	60.2	2.6	1.19	11.12	141	9.0	6.73	15.27	52.1
RBT	INT	96.3	63.7	3.0	1.14	10.89	137	7.3	6.96	15.13	66.4
RBT	INT	98.5	62.5	2.8	1.16	10.67	148	0.8	7.01	15.44	60.0
RBT	ENR	95.7	61.1	2.8	1.22	12.48	216	0.5	7.56	14.83	44.5
RBT	ENR	98.6	61.8	2.8	1.17	11.67	208	4.8	7.15	15.06	70.7
RBT	ENR	96.4	61.9	2.6	1.11	11.89	227	0.0	7.87	15.08	83.7
RBT	ENR	95.8	62.0	2.8	1.18	11.87	100	2.2	7.09	15.08	43.4