

AN ABSTRACT OF THE DISSERTATION OF

Neil F. Thompson for the degree of Doctor of Philosophy in Zoology presented on
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Title: Rearing Density as a Driver of Adaptation to Captivity and Traits under
Selection by Domestication in Hatchery Reared Steelhead (*Oncorhynchus mykiss*)

Abstract approved:

Michael S. Blouin

Releasing hatchery reared salmon and steelhead to supplement threatened and endangered populations is a widely used conservation tool. One issue with this strategy is hatchery fish have lower reproductive fitness than wild fish when spawning in the wild. One of the drivers of fitness loss in steelhead is adaptation to captivity via domestication selection. Although a cause of fitness loss is known, the mechanism and traits which domestication selection is acting on remain unknown. In a series of experiments using steelhead, (*Oncorhynchus mykiss*) I evaluated if: (1) high growth rate is being selected for in captivity; (2) if high levels of dominance correlate with fitness at release from the hatchery and (3) if reducing rearing density has an effect on the opportunity for domestication selection to act. I found that body size did not differ between first generation hatchery fish and wild fish reared in a common environment. Mother's body size and date of spawning was correlated with body size

of offspring in the hatchery. Larger mothers and earlier spawn dates resulted in larger offspring. I hypothesize that early spawned families had lower metabolic rates during embryonic development due to a chilling treatment that hatchery staff use to synchronize development across all spawning events. By reducing metabolic rate during development more energy could have been used for somatic growth compared to later spawned families. Hatchery juveniles are more aggressive than wild fish, but dominance level did not influence fitness in the hatchery environment in my experiments. A potential explanation for this result is that physiological characters that are correlated with dominance are truly under selection and dominance is simply a correlated response. The correlation between physiological characteristics (e.g. metabolic rate) and dominance may not be as strong in a novel environment, influencing the negative result. Lastly, I found lowering rearing density did not have an effect on the opportunity for selection to act in captivity. Equal amounts of variance in performance among families existed in high and low density. Additionally, rank order performance across densities was highly correlated (0.82-0.95 Spearman rank correlation) demonstrating that families that perform best in captivity will do so regardless of rearing density. Results from these experiments led to developing a novel model of how increased density might exacerbate domestication selection. In salmonids, the relationship between body size at release and probability of return tends to be strongly logistic, approaching truncation selection. Under high rearing density only the best pre-adapted families to captivity (having maladaptive traits for the wild environment) are able to grow above the threshold for high survival and return in large numbers to spawn. Families that are

poorly adapted to the hatchery (with traits that have higher fitness in the wild) do not attain the threshold body size and return in relatively small numbers to spawn.

Because the main effect of increased density is to shift the body size of all families downward, high density could simply reduce the number of families that are above the minimum body size to return, resulting in strong among-family selection.

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Rearing Density as a Driver of Adaptation to Captivity and Traits under Selection by
Domestication in Hatchery Reared Steelhead (*Oncorhynchus mykiss*)

by

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Neil F. Thompson, Author

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CONTRIBUTION OF AUTHORS

Chapter 2: Michael S. Blouin was involved in designing the experiments and editing the final manuscript. Melanie Marine and Mark Christie aided in genetic lab work and parentage analyses. Lyle Curtis and staff at the Oak Springs hatchery raised all fish for the experiment.

Chapter 3: Michael S. Blouin was involved in designing the experiment and editing the final manuscript. David L.G. Noakes contributed the research facilities used in the experiments.

Chapter 4: Michael S. Blouin was involved in designing the experiment and editing the final manuscript. David L.G. Noakes contributed the research facilities used in the experiments.

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CHAPTER 1
INTRODUCTION

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Wild Pacific salmon (*Oncorhynchus sp.*), have been in decline since the late 1800's and are currently at 1% of historic levels (National Research Council 1996). Historically, hatcheries were operated to increase the number of fish for fisheries, but recently with many populations of *Oncorhynchus* species listed under the endangered species act the focus of some hatcheries has shifted towards preventing extinction of specific populations (Williams *et al.* 1999). To supplement declining populations and provide harvest opportunities, approximately 4 billion hatchery salmon are produced and released annually in the North Pacific Ocean (Beamish *et al.* 1997). However, hatchery reared salmonids (Pacific and Atlantic) have reduced reproductive success (fitness) compared to wild-born individuals when spawning in the wild (Araki *et al.* 2007b, Thériault *et al.* 2011, Berntson *et al.* 2011, Hess *et al.* 2012, Ford *et al.* 2013, Milot *et al.* 2013). The reduced fitness of hatchery fish is a major concern as it has the potential to reduce fitness and productivity of populations when interbreeding occurs (Chilcote 2003, Araki *et al.* 2009, Chilcote *et al.* 2011). A wide range of causes, including genetic and environmental effects, have been shown to contribute to the reduced fitness of hatchery reared fish. However, the mechanisms and drivers of fitness loss remain unknown.

The reduction in fitness is not limited to a single *Oncorhynchus* species. Hatchery Chinook salmon (*O. tshawytscha*) from the Wenatchee River (Washington, USA) had 50 percent the reproductive success of their wild born counterparts (Williamson *et al.* 2010). Spawning location was found to explain a portion of the lower fitness estimate, but other factors such as genetic effects were likely acting to

reduce fitness. Ford *et al.* (2012) found reduced reproductive success of Chinook salmon in the Wenatchee River to be correlated with families that returned many young males as spawning adults. The reproductive success of young males in the wild was lower than the reproductive success of older males (Ford *et al.* 2012). The reduction in fitness of Chinook salmon appears to be driven in part by males that do not successfully produce returning offspring when spawning in the wild (Hess *et al.* 2012). Similarly, hatchery coho salmon (*O. kisutch*) from the Umpqua River (Oregon, USA) were found to have lower reproductive success than wild fish when released as smolts or unfed-fry (Thériault *et al.* 2011). Males had a stronger reduction in reproductive success compared to females and this pattern is also seen in Chinook (Thériault *et al.* 2011, Hess *et al.* 2012). Thériault suggests a relaxation of sexual selection due to artificial breeding as a potential cause of the loss of fitness, but could not rule out environmental or other genetic effects.

Steelhead (*O. mykiss*) have been studied the most extensively and much is known about the fitness difference between hatchery and wild fish populations. Araki *et al.* (2007a) found that hatchery winter run steelhead from the Hood River (Oregon, USA) had a 40 percent reduction in reproductive success compared to wild fish after a single generation of captive rearing. Other populations display a similar pattern of fitness reduction; hatchery steelhead in Little Sheep Creek (Oregon, USA) have 30-60% the reproductive success of wild fish (Berntson *et al.* 2011). The increasing evidence that hatchery and wild fish have differing fitness when reproducing in the wild has resulted in several hypotheses to explain these

differences. The strongest evidence that a genetic effect of fitness loss exists is that increasing hatchery ancestry reduces reproductive success in the wild. For example, a wild-born fish with one hatchery and one wild parent had 55% the reproductive success of a wild-born fish that had two wild parents (Araki *et al.* 2007b).

Furthermore, a wild-born fish with two hatchery parents had 37% the reproductive success of a wild-born fish with two wild parents (Araki *et al.* 2009). There is a possibility that environmental differences could contribute to the result of Araki *et al.* (2009) if, for example, fish with hatchery ancestry spawn in different parts of the river than fish with wild ancestry. However, the pattern of increasing hatchery ancestry reducing reproductive success in the wild environment is still present and a robust indication that genetic effects play a strong role in the fitness of steelhead. While environmental effects can't be ruled out completely in Chinook and coho, it is plausible that fitness loss may be heritable in those species as well.

Explanations for a heritable decline in fitness include: (1) domestication selection causing adaptation to captive rearing conditions, (2) relaxed natural selection and (3) heritable epigenetic effects. Blouin *et al.* (2010) found no evidence for large scale differences in DNA methylation between hatchery and wild steelhead from the Hood River (Oregon), although this does not rule out epigenetic effects. For relaxed selection to be effective in one or two generations there would need to be a massive genetic load present or a high mutation rate in the population. Otherwise, relaxed natural selection is a less-likely explanation for such rapid fitness loss. Domestication selection is the strongest candidate for explaining fitness loss in a

single generation and has been widely implicated in published literature with multiple signatures of domestication documented (Ford 2002, Araki *et al.* 2008, Ford *et al.* 2008, 2012, Christie *et al.* 2011).

Two signatures of domestication selection are present in Hood River winter steelhead (Christie *et al.* 2011). In 7 of 8 years examined, Hood River steelhead (all created using wild broodstock), displayed a strong performance tradeoff in which families that performed the best (returned many offspring as adults) in hatchery culture performed very poorly in the wild and vice versa (Christie *et al.* 2011). A tradeoff in performance demonstrates that certain phenotypes (families) have higher fitness in hatchery culture than in the wild environment, and that a single phenotype is not optimal in both environments. The second piece of evidence implicating domestication selection is that F1 broodstock (one generation of hatchery culture) returned almost double the number of adults than wild broodstock (zero generations of hatchery culture) in 4 of 5 years examined (Christie *et al.* 2011). Thus, one generation of hatchery culture significantly increased the performance of broodstock within the hatchery environment. This implies that certain traits are being inadvertently selected for in captive culture that increase performance in the hatchery. The single year that neither a performance tradeoff nor difference in adult returns existed between types of broodstock was when the hatchery fish were reared at a 7-fold lower density than in subsequent years. In that year, the juveniles were released at a larger body size as well, which is expected under lower density rearing conditions. Thus, rearing density may be a driver of domestication, with stronger

selection at higher rearing density. Because density influences growth rate and size at release, and because larger smolts return as adults at a higher rate than do smaller smolts (Ward and Slaney 1988, Ward *et al.* 1989, Henderson and Cass 1991, Tipping *et al.* 1995, Sogard 1997, Kallio-nyberg *et al.* 2004, Reisenbichler *et al.* 2008, Bond *et al.* 2008, Clarke *et al.* 2014, Kavanagh and Olson 2014, Osterback *et al.* 2014) growth rate (body size at release) could be a fitness related trait under selection in the hatchery that is causing the tradeoff in fitness. Thus, density (and selection for increased growth rate) is hypothesized to be a mechanism driving domestication selection and fitness loss in hatchery salmon.

Density also causes performance tradeoffs in other salmonid species. In Atlantic salmon (*Salmo salar*), mean family growth and survival at hatchery densities were negatively correlated with means for those traits at semi-natural densities (Saikkonen *et al.* 2011). Just as in the Hood River, we see a clear tradeoff in performance across hatchery and more nature-like conditions. Additionally, rearing at high density has a significant effect on other traits associated with fitness in salmonids. Salt water tolerance, predator avoidance behaviors, willingness to consume novel prey, dominance level and survival after release into streams were enhanced by rearing fish at low densities (Brockmark *et al.* 2007, 2010, Brockmark and Johnsson 2010). Any of these traits could be under selection in hatchery conditions if they increase performance in the hatchery. Taken together, these results suggest that rearing density in the hatchery can have major effects on traits associated

with fitness. We hypothesize that high rearing density has the *potential* to be a driver of domestication selection in hatcheries in many salmonid species.

While domestication selection has been implicated as a driver of fitness loss there have been no studies directly quantifying the drivers of adaptation to captivity, or the specific traits under selection. In Chapter 2, I test if selection for increased body size is occurring in the Hood River hatchery steelhead population. Growth rate is a compelling trait to be under strong selection for two reasons. First, body size at release is correlated with probability of return as an adult, and (2) hatchery fish are reared for a single year before smolting whereas wild fish take 1-3 years to reach smolt size (Quinn 2005). Combining the short captive rearing time and the survival advantage for larger fish at release, the selection pressure on growth rate may be quite strong.

In Chapter 3 I focus on the mechanism by which domestication selection may be acting in hatchery culture. Rearing density has major effects on fitness in hatchery conditions and has been shown to cause performance tradeoffs that may indicate that density is driving the ability for selection to occur. I hypothesized that by lowering rearing density, the opportunity for selection would be reduced. To accomplish this, I evaluated, (1) if a performance tradeoff existed within families across high and low densities, and (2) if there is increased variance in performance between families at high densities compared to low density (Figure 1.1). A performance tradeoff between densities indicates that phenotypes (families) that perform best in hatchery conditions (high density) do not perform well in more natural conditions (i.e. lower density)

(Figure 1.1). By determining if a tradeoff exists, I will be able to evaluate if a subset of phenotypes (families) are outperforming other families in high density conditions and being released with a fitness advantage. Testing for increased variation between families at high density allows me to determine if domestication selection is potentially stronger at high densities. Variation between families is critical for domestication to occur because selection needs variation to act upon. If there is little variation between families, then domestication selection has very little to act on and the selection pressure may be weak. If more variation is present between families at high density, the ability for domestication selection to act is increased. Understanding this would be of great value to hatchery managers because a major goal of conservation hatcheries is to reduce the effects of domestication as much as possible (Frankham 2008).

In Chapter 4 I sought to determine if dominance rank correlates with high fitness in captivity. Behavior, specifically dominance and aggression differ between hatchery and wild salmon (Weber and Fausch 2003). Hatchery fish are more aggressive and dominant individuals have higher food intake, increased growth rates and higher metabolic rates (Metcalf 1986, Abbott and Dill 1989, Metcalfe *et al.* 1995, Berejikian *et al.* 1996, Yamamoto *et al.* 1998). Given the advantages of being dominant I hypothesized families that are dominant and aggressive would be the largest at time of release from the hatchery. I also tested for the known effects of broodstock size and if ponding length (the mean fork length of a family a few weeks

after first feeding, immediately before being moved outdoors) correlated with juvenile fork length at the time of release.

In Chapter 5, I integrate the major results of Chapters 2-4 and suggest future directions for determining the drivers of differences in fitness between hatchery and wild salmonids. To further conservation efforts while continuing the use of hatchery reared fish, the drivers of adaptation and the traits that are best suited for high fitness in captivity need to be identified.

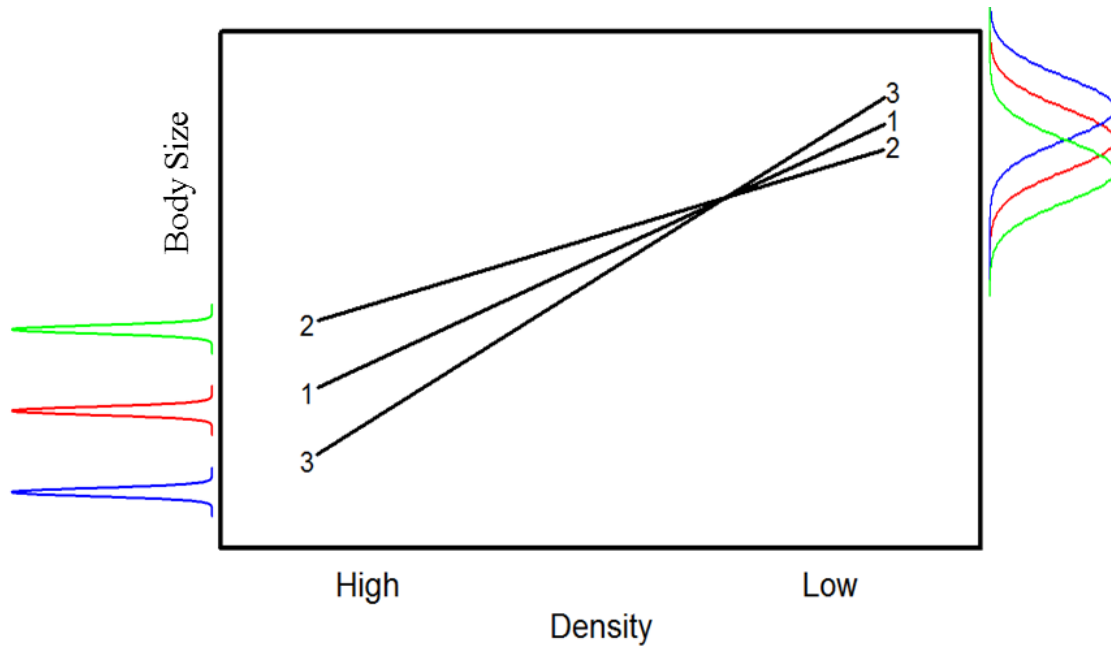


Figure 1.1

Hypothesized tradeoff in family performance and differences in among-family variance between density treatments. Each family is marked by a unique number and color. I expect a main effect of density such that fish raised at high density are smaller than fish reared at low density. However, I expect the among-family component of variance in growth to be increased at high density (illustrated with the normal distributions on each vertical axis). I also expect a family \times density interaction, such that different families perform best at different densities (crossed lines).

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CHAPTER 2

Growth rate does not differ between offspring of wild and first generation hatchery steelhead (*Oncorhynchus mykiss*) from the Hood River, Oregon during hatchery rearing

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Abstract

Conservation programs for Pacific salmon often spawn fish in captivity and rear their offspring in hatcheries, which substantially increases their survival rates. After a year of hatchery rearing, the juveniles are released into the wild in order to increase future adult population sizes. One challenge facing this strategy is that salmon and steelhead reared in hatcheries have lower reproductive success than wild fish when spawning in the wild. Recent studies have shown that one cause of the reduced fitness is genetic adaptation to the hatchery environment, but the traits under selection remain unknown. Hatchery reared fish are fed on an accelerated schedule to reach smolting in one year, whereas the majority of wild fish smolt at age 2 or older. Thus, inadvertent selection for fast growth may be occurring in captivity, and that genetic change could be leading to a loss of fitness post-release. We test if selection for fast growth rates and larger body size is occurring in a steelhead (*Oncorhynchus mykiss*) hatchery program. Using common garden experiments, we found no difference in body size between fish produced from wild and first-generation hatchery broodstock (either for 6 month old fish or for 1+ year old fish). Thus, our data show no evidence that Hood River steelhead have been under strong selection for faster growth rate. Potentially other traits were under selection, or a difference in growth rate was not apparent under the environmental conditions of our experiment. An environmental effect of the date a family of fish was spawned was found to have long lasting effects on body size. Families spawned early in the season had offspring that were larger than those families spawned later. A potential explanation for this is that

eggs from early-spawning families were chilled to ensure that all fish hatched on the same date. We hypothesize that the increased opportunity for more efficient somatic growth in the cooled families caused detectable differences in body size.

Introduction

Salmon and steelhead (*Oncorhynchus* spp.) raised in hatcheries often have lower fitness than wild-born fish when both spawn in the wild (reviewed in Berejikian and Ford 2004, Araki *et al.* 2008, Christie *et al.* 2014). Lower fitness of hatchery fish is a concern because the hatchery fish may reduce the fitness of the wild fish with which they interbreed (Chilcote 2003, Araki *et al.* 2007a, Chilcote *et al.* 2011). Domestication selection (i.e., genetic adaptation to the captive environment) has been implicated as one of the most likely causes of fitness loss in Pacific salmon (Ford 2002, Araki *et al.* 2008, Christie *et al.* 2011). However, the traits that are under selection in hatcheries remain unknown. If the traits that are responding to selection in the early stages of domestication can be identified, then it might be possible to modify the captive environment to reduce the loss of fitness.

Domestication is the response to selection for traits that maximize performance in captive environments, often at the expense of performance in other environments. In aquaculture, fast growth rate and large body size are deliberately selected for in a range of fish species including Atlantic (Gjedrem 1979, Fleming and Einum 1997, Fleming *et al.* 2002, Solberg *et al.* 2013), and Pacific salmonids (Gjedrem 1983, Hard and Hershberger 1995), which include steelhead *Oncorhynchus mykiss* (Gjerde and Schaeffer 1989, Hu *et al.* 2013, Devlin *et al.* 2013). For salmon raised for conservation purposes, increased growth rates may be selected for inadvertently due to size at release being correlated with post release survival, and thus the probability of returning as an adult (Ward *et al.* 1989, Henderson and Cass

1991, Sogard 1997, Tipping 1997, Zabel and Achord 2004, Reisenbichler *et al.* 2008, Bond *et al.* 2008, Clarke *et al.* 2014, Osterback *et al.* 2014). This hypothesis is made more compelling because hatchery steelhead spend 1 year in freshwater (in captivity) before going out to sea, whereas wild fish spend 1-3 years in freshwater, with the vast majority spending at least 2 years. Thus, it is plausible that inadvertent selection for fast growth is occurring in conservation salmon hatcheries and could be driving differences in fitness between hatchery and wild fish.

Strong selection for increased growth rate in hatcheries could explain the very rapid fitness declines observed when hatchery fish attempt to reproduce in the wild. Excessively high growth rate can be maladaptive in the wild, affecting traits such as juvenile survival (Arendt 1997, Sundstrom *et al.* 2004, Biro *et al.* 2004, Tymchuk *et al.* 2007), or perhaps even adult reproductive traits if early growth rate influences subsequent life history traits or behaviors such as egg weight in females and competitive ability in males during spawning (Jonsson *et al.* 1996, Fleming *et al.* 1997). Hatchery fish are known to show increased aggression and boldness, risk taking behaviors, reduced predator avoidance (Berejikian 1995, Berejikian *et al.* 1996, Fleming and Einum 1997, Tymchuk *et al.* 2006), and altered time of activity (nocturnal vs, diurnal) (Álvarez and Nicieza 2003). Trait changes such as these could be correlated responses to selection for the increased ability to grow quickly in the novel environment of a hatchery (i.e. abundant food, highly crowded, no predators).

A large body of data on winter run steelhead (*O. mykiss*) from the Hood River in Oregon shows that even a single generation of hatchery culture in this population

causes strong domestication, with concomitant loss of fitness in the wild (Araki *et al.* 2007b, Christie *et al.* 2011). To determine if increased growth rates and body size were being inadvertently selected for in this hatchery population, we conducted a series of common garden experiments using hatchery and wild Hood River steelhead. Specifically, our objective was to determine if fish having first-generation hatchery parents grew larger than fish having wild parents when both were raised in a common, hatchery environment.

Materials and Methods

Study site and Spawning Procedures

The Hood River supports a winter run of wild and first-generation hatchery steelhead that return from December to May. The majority of in-river spawning occurs from late April until mid-June, and broodstock for hatchery production are collected throughout this period. All hatchery fish are adipose fin clipped prior to release from the hatchery and identified by the missing adipose fin when returning as adults. Hatchery fish used as parents in this experiment were all first-generation (i.e. produced using wild broodstock parents).

Adult Hood River winter steelhead (*O. mykiss*) were captured in the fish passage facility at Powerdale Dam by the Oregon Department of Fish and Wildlife. Broodstock for the hatchery were transported to the Parkdale Fish Facility (operated by the Confederated Tribes of the Warm Springs) to be spawned. Collection of broodstock followed guidelines found in Reagan (Reagan 2010). When wild broodstock were collected, a similar number of hatchery broodstock were collected

for our experiment. Hatchery broodstock were identified by a missing adipose fin whereas wild broodstock had all fins intact. The treatment of hatchery and wild broodstock was identical throughout the spawning process. All fish were spawned at the Parkdale Fish Facility. Eggs were fertilized and water-hardened in an iodophor solution for approximately 1 hour before being transferred (on the same day) to the Oak Springs hatchery on the Deschutes River. Upon arrival at Oak Springs the embryos were transferred into vertical stack incubators until hatching and egg yolk absorption.

Once egg reserves were absorbed the juveniles were pooled and transferred to an indoor trough measuring 4.9x0.9x0.4 meters (length_xwidth_xdepth). Immediately after transfer to the trough, juveniles were fed with commercial fish food. Separate experiments were performed in 2009 and in 2010.

2009 Experimental Design

Single pair matings (one male and one female) were used to produce two fish types: HH were created using two hatchery fish, and WW were created using two wild fish. A total of 16 HH families and 19 WW families were produced. With this design (HH versus WW) we maximized the power to test for a main effect of fish type, with the obvious caveat that a significant effect could result from either additive genetic or maternal effects of parental type.

Each family was reared individually until exogenous feeding began, and then 15 individuals per family were pooled in indoor troughs on July 29, 2009. The families were pooled as soon as possible at initiation of exogenous feeding. Two

replicate troughs were created. One replicate was sampled in the fall and the second was sampled in the spring. We chose to have an early sample and late sample because we wanted to compare the results from early and late growth to see if similar factors (e.g. parent type) predicted juvenile growth throughout development.

The first replicate (fall sampling) was reared in the indoor trough until sampling on October 20, 2009 (92 days of growth). The spring replicate was moved outdoors to a concrete tank in late October when the first replicate was sampled. The outdoor tank was a circular concrete tank measuring 9.1 meters in diameter and 0.9 meters deep. Feeding schedules and pond maintenance followed standard procedures used for the production class of Hood River winter steelhead (Olsen 2007, Reagan 2010), although the fish were raised at much lower density than in the production tanks.

At the time of sampling all fish were euthanized in a solution of MS-222 according to guidelines found in AVMA (Leary *et al.* 2013). Each fish was measured for fork length (millimeters), and a fin tissue sample was stored in 95% ethanol for assigning individuals back to their respective families via genetic parentage analysis. We used fork length for all length measurements because total length is affected by fin degradation associated with hatchery rearing and does not provide as accurate an estimate of body length.

2010 Experimental Design

In 2010 we repeated the experiment with three main changes. First, we created reciprocal crosses within and among fish types in order to be able to

distinguish maternal versus additive genetic effects. Second, we used only a single replicate and sampled them in the spring. Third, we reared fish in a smaller enclosure than in 2009 in order to make the rearing density higher in order to more closely mimic conditions experienced by the production class of fish.

A series of 2_x2 matrices were used to spawn broodstock, each matrix consisting of one hatchery and one wild male crossed factorially with one hatchery and one wild female. This produced four fish types in each matrix, where the mother is listed first: (1) wild_xwild (WW), (2) wild_xhatchery (WH), (3) hatchery_xwild (HW) and (4) hatchery_xhatchery (HH). We created 18 of these 2x2 matrices.

Each full-sibling family was reared independently until exogenous feeding began. Fifteen juveniles per family were then randomly chosen for the experiment and pooled together. All juveniles were transferred from incubators to the indoor trough on July 19, 2010. The same feeding and maintenance schedules were used as in the 2009 experiment. Transfer from the indoor trough to an outdoor 6_x10_x2.5 foot screen enclosure inside a concrete raceway occurred in early January 2011. Fish were reared until April 28, 2011 at which point the experiment was terminated and all fish were measured. Fork length was measured (mm) and a fin tissue sample was taken for parentage analysis just as in 2009.

Genetic Parentage Analysis

Assigning the fish back to their parents was performed via genetic parentage analysis. DNA was extracted using Chelex 100 (Nelson *et al.* 1998). Six polymorphic microsatellite loci from the SPAN B suite were amplified via PCR

(Stephenson *et al.* 2009). All six microsatellites were multiplexed and amplified in a single reaction. Genotype scoring was performed on an ABI 3730 capillary electrophoresis system (Applied Biosystems, Foster City, California) at the Oregon State University Center for Genome Research and Biocomputing. GeneMapper version 4.1 (Applied Biosystems, Foster City, California) was used to analyze genotype data. The parentage analysis software SOLOMON was used to assign juveniles into putative families using the exclusion method as broodstock pairings were known (Christie *et al.* 2013).

Statistical Analysis

A linear mixed effects model was used to determine if hatchery fish grew larger than wild fish in 2009 and 2010. To assess if a mixed model better fit the data than an ordinary linear regression, a likelihood ratio test was performed. After deciding on the optimal variance structure, all fixed effects were evaluated with likelihood ratio tests. The final linear mixed model was fit with restricted maximum likelihood (REML). We chose to use a mixed model because strong family effects could be present that would potentially mask a difference in growth rate between hatchery and wild types if left unaccounted. All model selection was done following protocols of (Zuur *et al.* 2009) using the nlme package in R version 2.15.1 (Pinheiro *et al.* 2012, R Core Team 2012)

The model used in 2009 was Fork Length ~ Fish Type +Spawn Date+ Mother Fork Length + Father Fork Length + Fish Type*Spawn Date + Fish Type*Mother Fork Length + Fish Type*Father Fork Length + Mother Fork Length*Father Fork

Length + Family (random factor). All interactions involving fish type were included to allow for different effects of the covariates for hatchery and wild type fish. The interaction between Mother Fork Length and Father Fork Length was included to account for non-additive effects of parent size. In 2010 the same model was used except we broke fish type into mother type and father type to be able to determine the effects of the reciprocal crosses. To account for the non-independence of the 4 fish types in the 2010 experiment we also performed the analysis with only WW and HH fish. No difference was found in the results from the subsetting analysis (WW and HH only) compared to the full analysis (all 4 fish types). Thus, we only report results from the analysis with all 4 fish types included.

For the 2010 experiment we also used paired t-tests to determine if a genetic effect of hatchery ancestry increases growth rate within a mother type (i.e. comparing half siblings that share a common mother but differ in father type). This analysis tests for an additive genetic effect of fish type, as it removes any possible maternal effects of fish type. We performed the test twice, once using hatchery mothers and once using wild mothers. Both analyses tested for differences in body size between half-siblings (shared mother) that differed only in type of father (hatchery versus wild).

Results

2009 fall sampling

A mixed model with family as a random factor was preferred over a fixed effects model (Likelihood ratio test, $P = < 0.001$). Fish type (i.e. HH vs. WW) had no effect on body size (95% CI [-0.008, 0.410] cm, with wild fish being slightly larger

than hatchery fish; linear mixed effects model, $P = 0.06$). The only significant predictor was spawn date where being spawned later decreased body size (Figure 1, linear mixed effects model, $P = 0.005$). For every 30 days after the first spawning event, fork length is reduced by 0.28 cm (95% CI [-0.09, -0.49] cm). For results of likelihood ratio tests on non-significant fixed effects see Table 1.

2009 spring sampling

As before, the mixed linear model was chosen over a fixed effects model (Likelihood ratio test, $P = <0.001$). Fish type did not affect juvenile body size (95% CI [-0.62, 1.38] cm with wild as reference group, linear mixed effects model, $P = 0.45$). Again, individuals that were spawned later in the season were shorter than those spawned early in the season (Figure 1, linear mixed effects model, $P < 0.001$). On average, being spawned a month after the first spawning event reduced body size by 1.69 cm (95% CI [0.79, 2.59] cm). An interaction effect between fish type and mother fork length indicated that the effect of mother fork length on offspring size differed for hatchery and wild type fish (linear mixed effects model, $P = 0.027$). A positive relationship is seen between mother fork length and juvenile body size for wild fish whereas a negative slope is found for hatchery fish (Figure 2). However, the 92 cm hatchery mother appears to be driving this result (Figure 2). Without the 92 cm mother there is no interaction (likelihood ratio test, $P = 0.62$). For results of non-significant effects see Table 1.

2010 results

The mixed linear model was chosen over a fixed effects model (Likelihood ratio test, $P = <0.001$). No interactions were significant in the analysis (Table 1). The magnitude of the fixed effects did not vary with hatchery or wild fish types, and non-additive effects of parent length were not present (Table 1). Neither mother type nor father type affected juvenile fork length (95% CI [-2.6, 7.4] and [-2.7, 7.3], respectively, linear mixed effects model, $P = 0.34$ for mother type, $P = 0.36$ for father type). No effect of spawn date was found (95% CI [-0.30, 0.24] cm; linear mixed effects model, $P = 0.83$), but the spawning season was only 34 days long which was shorter than the 47 day long spawning season in 2009. Mother's fork length had a slight positive relationship with juvenile fork length (linear mixed effects model, $P = 0.05$, Figure 3). An increase of 10 cm in mothers length increased juvenile length by 0.88 cm (95% CI [-0.01, 1.78]). Father's fork length did not affect juvenile fork length (linear mixed effects model, $P = 0.53$)

No difference in fork length was found in fish that shared a mother type but differed in having a hatchery or wild type father (paired t-test for within wild and hatchery mothers respectively, $P = 0.50, 0.56$). Thus, no additive genetic effects of hatchery *versus* wild ancestry were apparent in this data set (Figure 4).

Discussion

We found no difference in body size between juveniles having wild or hatchery parents, when raised in a common hatchery environment. Thus, we do not see evidence for the hypothesis that domestication in the Hood River steelhead

hatchery program has involved selecting for faster growth rates in the hatchery. There are several explanations for this result. First, domestication in this population may simply involve response to selection on traits other than body size at release. Alternatively, it is possible that body size is under selection but we did not see the effect in our experiment. One reason this could occur is because our experiments did not fully replicate the environment experienced by fish in the main production tanks at the hatchery. In 2009 our fish were reared at much lower densities than experienced by fish in the production tanks. In 2010 we crowded them into a screened enclosure within a concrete raceway in order to try to replicate that density, but we could not replicate the experience of being in a production tank with ~50,000 other fish. Thus, we cannot rule out a genotype-by-environment interaction in which a difference in body size would be expressed under the presumably more stressful environment of a production tank. For example, differences between inbred and outbred offspring are often strongly apparent in stressful environments but weak or undetectable in benign environments (e.g. (Crnokrak and Roff 1999)). Thus, although our results argue against the hypothesis that the hatchery inadvertently selected for high juvenile growth rate, we cannot completely rule it out.

We did find what appears to be an environmental effect of hatchery rearing that could have significant and long lasting effects on juvenile body size. A protracted spawning season occurred in 2009. Families that were spawned earlier in the season grew larger than those that were spawned later. Female broodstock spawned at different times did not differ in fork length (linear regression, $t = 0.317$, P

= 0.75), so the correlation between juvenile body size and spawn date is not likely due to genetic or maternal effects of female body size. In 2010 there was no correlation between spawn date and offspring size, but in 2010 the spawning season was shorter by 2 weeks. Furthermore, the majority of families produced for the 2010 experiment were fertilized over a 14 day period at the end of the spawning season leaving little time for effects of spawn date, if present, to accumulate.

A potential mechanism to account for the correlation between spawn date and offspring size is that Oak Springs hatchery staff reduce water temperature of early spawned families to synchronize development between embryos from all spawning events. This common practice ensures that all embryos hatch and begin exogenously feeding at the same time. Thus, early-spawned embryos spent much longer developing very slowly at cold temperatures than late-spawned embryos. Early-spawned embryos could thus have expended less energy for metabolism and used the surplus for somatic growth (Hamor and Garside 1977, Heming 1982, Ojanguren *et al.* 1999). Later spawned families experienced higher temperatures and a potentially higher metabolic cost which can reduce the amount of energy available for somatic growth. Surprisingly, the effect of spawn date was still detectable at the spring sampling nearly a year after fertilization. This result demonstrates how environmental effects can inadvertently give an edge to a portion of the production class throughout their entire time in the hatchery. Such an environmental effect could partly explain the high variance in numbers of returning fish per hatchery family observed in this and other hatchery programs (Christie *et al.* 2012), if size at release

has a strong effect on survival (Ward *et al.* 1989, Holtby *et al.* 1990, Henderson and Cass 1991, Tipping 1997, Zabel and Achord 2004, Reisenbichler *et al.* 2008, Bond *et al.* 2008, Duffy and Beauchamp 2011, Woodson *et al.* 2013, Clarke *et al.* 2014, Osterback *et al.* 2014).

The size of a juvenile's mother was positively correlated with juvenile body size in 2010. Additive genetic effects on body size have been well documented in salmonids (Gjederm 1979). However, the lack of significant effects of father body size in 2009 and 2010, and no effect of mother size in 2009, argue against strong additive genetic effects of parent size on juvenile size under these experimental conditions. Egg size could have played a role in the 2010 result because mother fork length and egg size were correlated. Perhaps the effect of chilling embryos in 2009 (spawn date effect) muddled the additive genetic effect if one was present during 2009.

In summary, we found no difference in growth rate between the offspring of wild fish and first-generation hatchery fish that were raised in a common, hatchery environment. Thus, we see no evidence to support the hypothesis that domestication in Hood River *O. mykiss* has involved inadvertent selection for high growth rates in hatchery culture. On the other hand, experimentally mimicking the environment experienced by the production class of fish is extremely challenging, so we cannot rule out that a difference would have been expressed under true production conditions. An ideal test of the growth rate hypothesis would involve sampling an

actual production class in which individuals differing in hatchery ancestry were reared in a common environment.

An interesting and unexpected effect that we observed in 2009 was that spawn date was negatively correlated with family body size (early-spawned embryos were larger, even a year later). No phenotypic traits that we could measure on the parents, such as run date or body size, explained the result. One possible explanation is the common practice of cooling early-spawned embryos in order to insure that all families hatch at the same time. That opportunity for more efficient somatic growth during embryonic growth could translate into detectable differences in body size at release is impressive. One interesting consequence of such a purely environmental effect is that it could generate non-random variance among families in numbers of returning fish if size at release is correlated with survival.

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Table 2.1

F values and associated *P*-values for all non-significant effects from likelihood ratio tests.

		Predictor	F value	<i>p</i> -value
2009	Fall	Type*Mom length	0.31	0.57
		Type*Father length	0.65	0.42
		Type*Spawning date	0.02	0.89
		Mom length*Father length	0.07	0.79
		Mother length	1.49	0.22
		Father length	1.90	0.17
2009	Spring	Type*Father length	0.12	0.73
		Type*Spawning date	0.87	0.34
		Mom length*Father length	4.45	0.03
		Father length	1.34	0.25
2010	Spring	Mom type*Mom length	1.66	0.64
		Dad type*Father length	1.64	0.65
		Mom type*Spawning date	0.66	0.72
		Dad type*Spawning date	0.32	0.85
		Mom length*Father length	1.41	0.23
		Mother length	1.49	0.22
		Father length	1.90	0.17

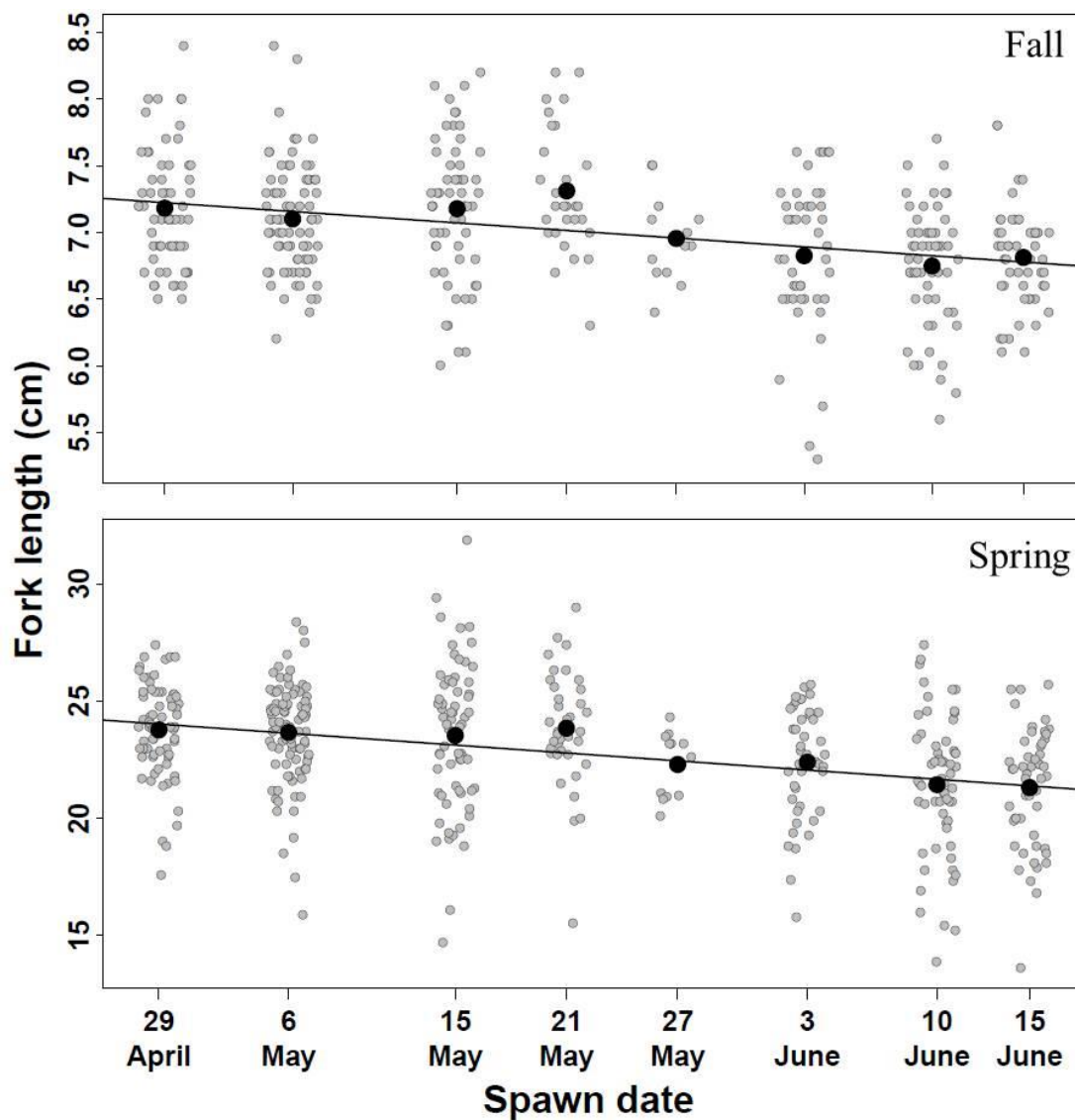


Fig 2.1

Correlation of spawn date and fork length from the fall and spring samples during 2009. The mean fork length for all fish within a spawn date is represented by a large black circle. A later spawn date reduced fork length at both sampling events. Some spawn dates have more data because different numbers of families were created on each spawn date and points represent the offspring from all families (both hatchery and wild).

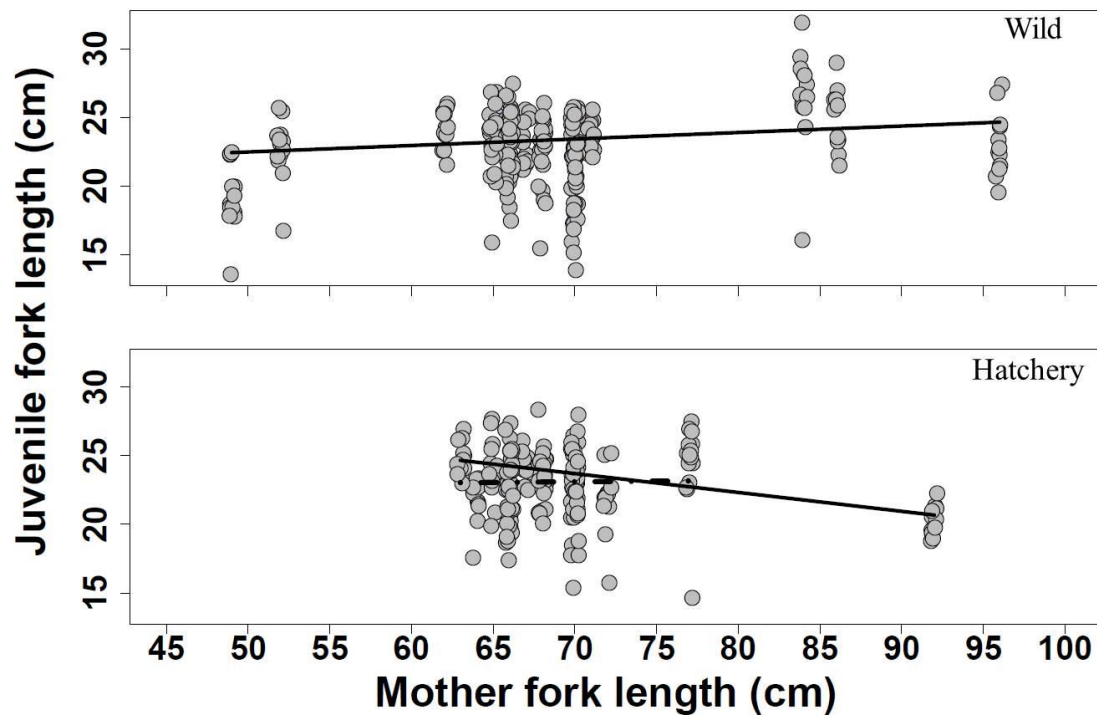


Figure 2.2

Correlation of mother's fork length on juvenile fork length for wild and hatchery juveniles at the spring sampling in 2009. Each position on the X-axis is a mother's length, and the points above her length are the lengths of her offspring. The solid lines are the linear regression estimate of the mother fork length variable for each fish type. The 92 cm hatchery mother is driving the negative effect of mother fork length on juvenile fork length, and thus the fish type by mother fork length interaction. The dashed line is the linear regression estimate of mother fork length without the 92cm female. A slight positive slope is present without the 92 cm hatchery mother.

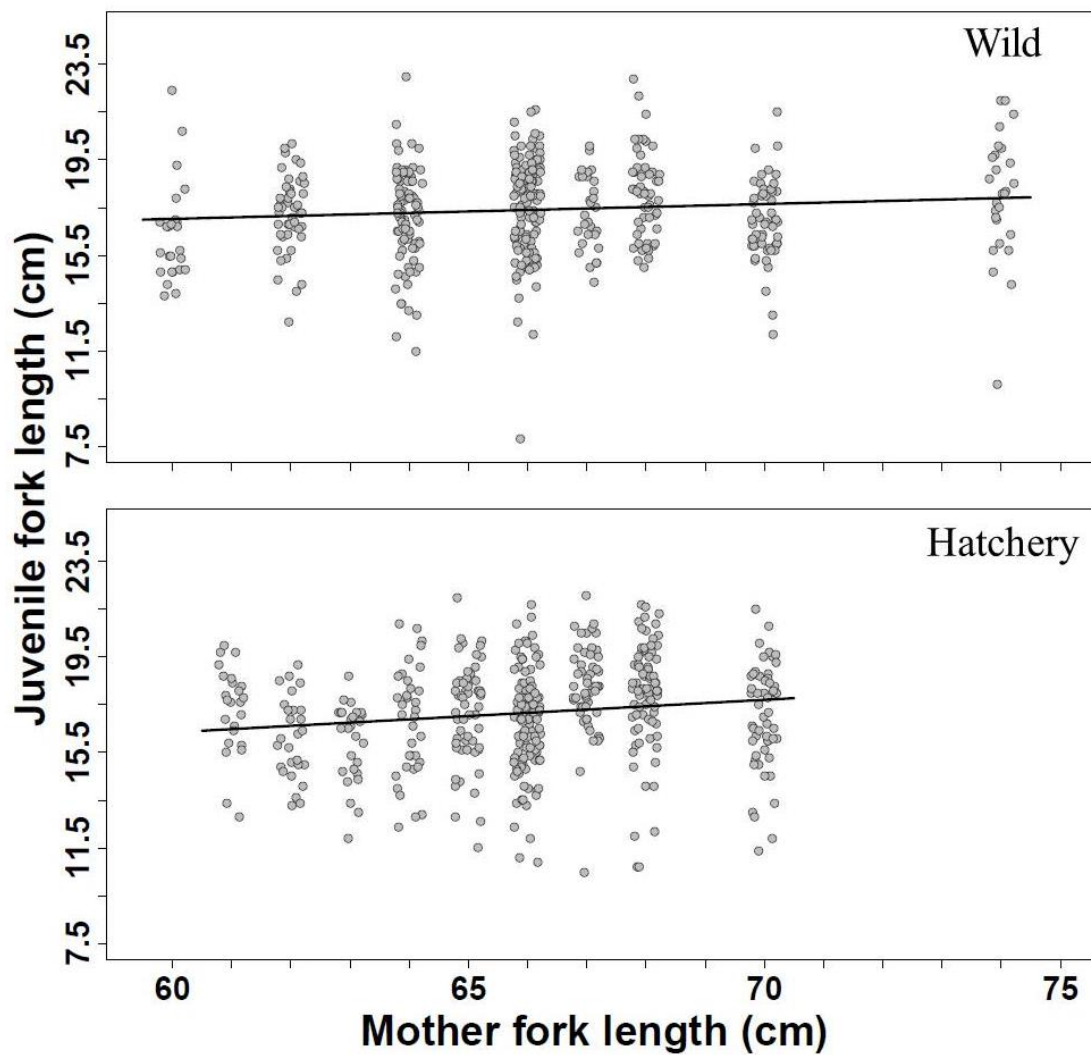


Figure 2.3

The correlation of mother's fork length with juvenile fork length for wild and hatchery fish in 2010. There was a statistically significant relationship in which larger females produced larger offspring. Multiple mothers had the same fork length so points are jittered to show all data. No relationship between juvenile fork length and father size was observed.

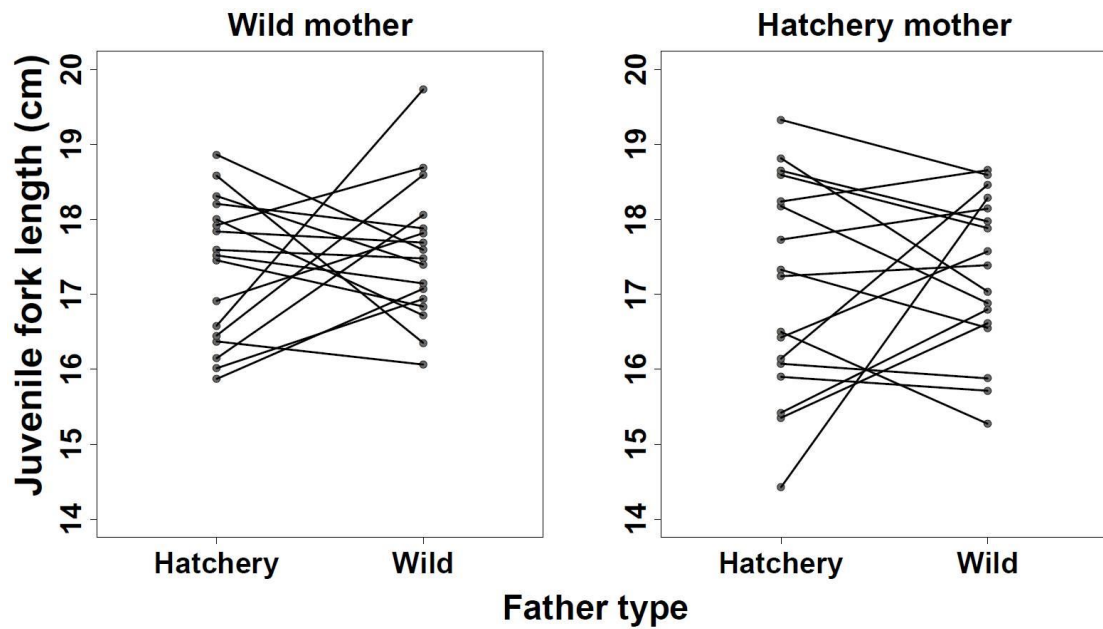


Figure 2.4

Mean body size of half-sibling families. The half-sibling analysis examined the effect of father type within a common maternal type. This analysis tested for additive genetic effects of father type. Notice that there is no consistent pattern as lines connecting half-sibling families have both positive and negative slopes. If increased growth rate was being selected for in hatchery fish, we would see negative slopes in both plots.

CHAPTER 3

The effect of high rearing density on signatures of domestication selection in hatchery culture of steelhead (*Oncorhynchus mykiss*)

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Evolutionary Applications

To be submitted

Abstract

The release of hatchery-reared steelhead (*Oncorhynchus mykiss*) to supplement threatened and endangered stocks is a common conservation tool used in the Pacific Northwest. Yet, conservation program-hatchery fish often have lower fitness than wild fish when spawning in the wild. This loss of fitness is known in part to be due to the rapid response to domestication selection. However, the drivers of domestication in captivity are unknown. Size at release is correlated with survival at sea. Therefore, we hypothesize that high rearing densities typical of hatchery rearing could exacerbate domestication in two ways. First, if high density increases the among-family variance in performance (growth rate in the hatchery), then the opportunity for among-family selection on body size after release could be enhanced. Second, if there is a strong genotype-by-environment (family-by-density) interaction such that rank-order family performance differs between low and high (presumably less natural) densities, then high density could cause selection in favor of families that would have performed less well in the wild. We used steelhead from the Siletz River (Oregon, USA) to test these hypotheses. There were strong main effects of density (high density reduced body size), but high density did not increase the among-family component of variance in body size (i.e. the intraclass correlation). Similarly, there were strong main effects of family, with 33-53% of the total variation in size accounted for by family identity. No strong genotype-by-density interaction was observed. We see no strong evidence that high density changes either the rank-order performance of families or the proportion of variance in body size that is distributed

among-families. Thus, we propose an alternate model of how increased density might exacerbate domestication selection. In salmonids, the relationship between body size at release and probability of return tends to be strongly logistic, approaching truncation selection. Because the main effect of increased density is to shift the body size of all families downward, high density could simply reduce the number of families that are above the minimum body size to return, resulting in strong among-family selection.

Introduction

Release of captively reared salmonids is a widely used tool to conserve threatened and endangered populations (Waples and Drake 2004). One issue with this conservation strategy is that hatchery reared salmon and steelhead often have lower fitness than wild fish when spawning in the wild environment (for a review: Christie *et al.* 2014a). The effect of captive rearing on fitness is rapid, with first-generation fish (i.e. from wild broodstock) having markedly reduced fitness compared to their wild born counterparts (Christie *et al.* 2011). Environmental effects do contribute to the differences in fitness, but they do not account for them fully (Williamson *et al.* 2010). There is strong evidence that intense domestication selection via adaptation to captivity is occurring and contributing to fitness loss in the wild (Christie *et al.* 2011). However, we do not know the traits under selection or the environmental conditions in the hatchery driving the strong selection. Identifying what environmental conditions in captivity are causing domestication selection may point to a way to lessen the selection pressure and reduce fitness loss in the wild.

Strong evidence of rapid adaptation to the hatchery was observed in Hood River steelhead (*Oncorhynchus mykiss*) (Christie *et al.* 2011). First, a strong tradeoff was found in which families that performed well in the hatchery (returned many adults) performed poorly when spawning in the wild, and vice versa. Second, while first-generation hatchery fish performed worse than wild fish in the wild, they outperformed wild fish when used as broodstock in the hatchery. Another signature of selection seen in hatchery steelhead is that the number of returning fish per

hatchery family is highly skewed, whereby some families return many offspring, while others return few or no offspring (Christie *et al.* 2014b). This result suggests a subset of families are better pre-adapted to captivity than others.

Christie *et al.* (2011) noticed an interesting correlation in that the signature of domestication in Hood River steelhead was strongest in cohorts that were raised at the highest densities. Thus, we hypothesize that rearing density influences the strength of domestication selection. Rearing salmonids at high density is known to reduce growth rates (Refstie 1977, Banks 1992, Kavanagh and Olson 2014), influence behavioral interactions (Riley *et al.* 2009), and increase wounding and fin erosion (Kavanagh and Olson 2014), all of which may contribute to differences in family performance during the course of captive rearing. At release from the hatchery, body size is known to be correlated with probability of return as an adult for steelhead and Pacific salmon (Ward *et al.* 1989, Henderson and Cass 1991, Sogard 1997, Tipping 1997, Zabel and Achord 2004, Reisenbichler *et al.* 2008, Bond *et al.* 2008, Clarke *et al.* 2014, Osterback *et al.* 2014). Thus, body size at release is an important component of fitness with selection likely acting for increased body size in hatchery reared fish.

We propose two ways in which high rearing density could exacerbate rapid domestication and loss of fitness in the wild. First, if high density increases the among-family variation in performance in the hatchery, then the opportunity for among-family selection would increase (Figure 3.1). Second, if high density substantially changes the rank order performance of families, then high density might

favor families having traits that are less favorable in the wild (here we presume that low-density conditions are more like the natural environment than high-density conditions). Genotype (family) by environment (density) interactions are ubiquitous and can have strong effects on fitness in novel environments (Via and Lande 1985). If the traits that confer fast growth in low density are different from those traits that produce fast growth in high density, a significant interaction is expected.

In this study we reared steelhead (*O. mykiss*) under high and low density conditions to determine if (1) high density rearing increases among-family variation in performance (increased opportunity for selection among families) and (2) if a genotype-by-environment interaction occurs such that rank order family performance changes substantially. We expected at high density the opportunity for selection to be larger than at low density. We also hypothesized a significant genotype-by-environment interaction would exist across densities such that the best performing families in high density would not be the best performing families in low density. We chose to use steelhead as our study model because the strongest evidence for effects of domestication is seen in this species.

Materials and Methods

Sample collection and spawning procedures

Mature winter steelhead were collected using a fish trap located at river mile 64.5 on the Siletz River by the Oregon Department of Fish and Wildlife (ODFW). Following standard ODFW procedures, broodstock were transported to the Alsea River hatchery and artificially spawned (Oregon Department of Fish and Wildlife

2013). All matings were 1 to 1 pairings with no individual being used in more than one mating. Winter steelhead return over the span of 2 calendar years (typical return is December to May) and spawn in the spring. For example, adults that returned in late 2011 and early 2012 are referred to as the 2011 run year, and their offspring that were born in spring of 2012 are referred to as the 2012 brood year. For simplicity, we here use brood year to identify each cohort of offspring and their parents.

The experiment was conducted in each of two years. In 2012, the broodstock (parents of our experimental fish) were first-generation (F1) hatchery fish (i.e. they had wild parents, but spent their juvenile phase in the hatchery). In 2013, all broodstock were wild individuals, as determined by the presence of the adipose fin. A fin clip was taken from each broodstock fish and stored in 95% ethanol for subsequent parentage analysis.

After fertilization and water hardening all embryos were transported to the Oregon Hatchery Research Center (OHRC) in Alsea, Oregon. All embryos were kept in separate family groups until approximately two weeks after first exogenous feeding.

Density treatments

Our goal for density treatments was to have approximately 140 fish/m³ in the high density and 20 fish/m³ in the low density, which mirror the range of densities experienced by fish in the Hood River hatchery program. Fry were randomly chosen from each family and put into one of two treatments. The high density treatment was set up in 1.8 meter diameter tanks while the low density treatment was set up in 3.6

meter diameter tanks. All tanks were tan fiberglass with netting covering the surface to provide shade, cover and protection from avian predation. We note that density is confounded with tank size in this experiment. Tank limitations at the research center did not allow a test using same sized tanks with varied number of individuals per tank.

In 2012, six families were used and each family had 35 juveniles per tank for both treatments. Two replicates of each density were created on August 7, 2012. No marking of the fish was done prior to termination of the experiment.

In 2013, 10 families were used with each family contributing 20 fish to high density treatments and 31 fish to low density treatments. On July 15, 2013 juveniles were moved from the indoor incubation room to the outdoor tank farm, and three replicates of each density treatment were created. All individuals had the adipose fin removed during January 2014 following ODFW procedures to mimic standard hatchery production as closely as possible (Oregon Department of Fish and Wildlife 2013).

The density treatments differed slightly between years due to loss of 4 families in 2012. A mechanical malfunction occurred in the hatchery during early rearing (when each family was being reared independently) which led to the loss of 4 families. We attempted to get as close to the target densities for our treatments as possible, but do note a slight difference between years.

Fish husbandry

Individuals in all treatments were fed to satiation daily with Bio-Oregon commercial fish feed. This included 6-8 feedings per day until fish reached 0.75 grams; 4-6 feedings until the fish were 3 grams then 2-4 feedings per day for the remainder of the experiment (R. Couture, Oregon Department of Fish and Wildlife, personal communication). Rearing procedures including tank cleaning and health monitoring followed the Oregon Department of Fish and Wildlife Alsea hatchery operations plan for Siletz stock winter steelhead (Oregon Department of Fish and Wildlife 2013).

Sampling

In the 2012 experiment, fish were raised in outdoor tanks for four months before sampling. In 2013, fish were raised for a full 9 months outdoors (to full release size). At sampling, all fish were euthanized via overdose of MS-222 following procedures outlined by the American Veterinary Medical Association (Leary *et al.* 2013). Fork length was measured in millimeters for each individual and a fin clip was taken for genetic parentage analysis. Fin clips were stored in 95% ethanol.

Genetic parentage analysis

Genotypes at 6 microsatellite loci were used to assign juveniles back to family groups. DNA was extracted using Chelex 100 and followed protocols of Nelson *et al.* (1998). The SPAN B suite of loci (*Ogo4*, *Omm1046*, *Omy7*, *One102*, *Ots4*, *Ssa407MP*) were amplified using the following PCR thermal cycling regime: 95°C for 15 minutes, 35 cycles of 94 °C for 30 seconds, 57 °C for 90 seconds, 72 °C for 60

seconds with a final extension of 60 °C for 30 minutes (Stephenson *et al.* 2009). All loci were multiplexed in a single reaction for each fish. Genotype scoring was performed on an ABI 3730 capillary electrophoresis system (Applied Biosystems, Foster City, California) at the Oregon State University Center for Genome Research and Biocomputing. GeneMapper version 4.1 (Applied Biosystems, Foster City, California) was used to analyze genotype data.

To perform parentage analysis we used the SOLOMON program with an exclusion method because broodstock pairings were known (Christie *et al.* 2013). Juveniles that mismatched at any loci were checked manually to determine the putative broodstock pairing they belonged to. All fish were eventually assigned back to a known broodstock pair.

Statistical analysis

To assess if high density rearing increased variation in among-family performance we calculated the intraclass correlation (ICC) for final body size in each tank. The ICC is a ratio of variance among families to total variance within each tank (sum of variance among and within families). A large ICC value would suggest that the opportunity for among-family domestication selection to act is large (bottom panel Figure 3.1). ICCest in the ICC package of R was used to calculate ICC values and variance components (R Core Team 2012). A Welch's t-test was used to determine if the ICC values from low and high density tanks differed statistically.

A linear mixed effects model was used to determine if a significant family-by-density (genotype-by-environment) interaction occurred. Our response was mean

family fork length. The model included fixed terms for family, density and the interaction between family and density as well as a random term for tank. The random tank term accounts for correlation between families within a tank as well as tank to tank variation. All mixed modeling was done following protocols of Zuur *et al.* (2009) using the nlme package in R version 2.15.1 (Pinheiro *et al.* 2012, R Core Team 2012)

Results

Opportunity for selection

Intraclass correlation (ICC) values for 2012 and 2013 can be found in Table 1. No significant difference in ICC values was found between high and low density tanks in 2012 (Welch's t-test, $t = -3.9$, $P = 0.16$) or in 2013 (Welch's t-test, $t = -0.92$, $P = 0.42$). ICC values were much more variable among high-density tanks than among low density tanks (Table 1). The proportion of variance between families appeared to increase with longer time in captivity as 33-56% of total variance in 2013 was due to differences between families compared to 14-19% for the shorter 2012 experiment.

Performance tradeoffs

In 2012 strong and significant effects of family ($F(5,10) = 4.1$, $P = <0.0001$) and density ($F(1,2) = 37.7$, $P = 0.02$) were present on juvenile body size. All families grew slower at high density. However, the interaction between family and density was nonsignificant ($F(5,10) = 0.52$, $P = 0.76$) suggesting that the effect of density did not vary substantially across families.

Strong and significant effects of family ($F(1,4) = 37.4$, $P = 0.004$), and density ($F(9,36) = 60.0$, $P = <0.0001$) were again observed in 2013. A significant family-by-density interaction ($F(9,36) = 3.1$, $P = <0.008$) was also observed in 2013. The significant interaction result indicates that the effect of density was not consistent across families with some exhibiting larger changes in mean body size compared to others. However, the actual magnitude of the interaction effect is small relative to the main effects of family and density (Figure 3.2). The interaction accounted for 2 percent of the total variance whereas density and family accounted for 43 and 47 percent, respectively. Furthermore, when the rank order performance of families is compared across density treatments the correlations are high, ranging from 0.82 to 0.95 (9 pair-wise Spearman rank correlation coefficient between each pair of high and low tanks; Table 2). The high correlation of rank order performance between density treatments also suggests that rank order performance of families changes very little from low to high density.

Discussion

In this study we found that reducing rearing density had little effect on diminishing differences in among-family performance and presumably, the ability for selection to act on traits that enhance growth rate in the novel, hatchery environment. We also saw no evidence for strong genotype-by-environment interactions across densities. We note that density was confounded with tank size in our study and could have affected our results. If the opportunity for selection and genotype-by-environment effects are influenced by the number of interactions between fish to a

greater degree than rearing density, per se, this may have resulted in our experiments not supporting our hypothesis.

A statistically significant interaction was found in only the second year of our study, and the effect was small relative to the large main effects of family and density. Spearman rank correlations between mean family size across treatments are quite high (range of 0.82-0.95) demonstrating that the rank order performance changes very little from low to high density. It appears that the families that are best pre-adapted to captivity will perform at a high level irrespective of the rearing density, while those families with maladaptive traits for life in the hatchery will do poorly regardless of the rearing density.

A surprisingly large amount of the total variation is explained by among-family differences, with as much as 33-53% being due to family level effects in 1 year old fish. Even when fish are sampled at a small body size (fork length range: 64-119 mm) 14-19% of the variation present is due to among-family effects. Under standard rearing protocols it appears that large differences in pre-release fitness are present among families. Thus, because selection acts on variance in fitness, there is the potential for strong selection regardless of the rearing density within the hatchery.

A possible model for how increased density might increase the strength of domestication selection

Christie *et al.* (2011) noticed a strong correlation between rearing density of cohorts of steelhead and an indicator of domestication in each cohort (the tradeoff

between family performance in the hatchery *versus* in the wild). Yet, here we saw no evidence that increased density increases the opportunity for among-family selection on traits that enhance growth rate in the hatchery. Nor does changing density appreciably influence which families perform best. So, if increased density does enhance domestication selection, how might that occur?

An alternate model for how increased density could exacerbate domestication selection relies on three observations. (1) The effects of family identity on body size at release are huge. As seen in our study 33-53% of the total variance can be explained simply by family identity in one-year-old fish. Thus, selection has a large amount of variation, owing only to family identity, to act on when steelhead are reared in hatcheries. Furthermore, the heritability of growth rate in salmonids is typically in the range of 0.16 to 0.6 (Gjedrem 1983, Gjerde and Schaeffer 1989, Hu *et al.* 2013). Thus, although our study used full-sibling families, we expect that a large proportion of the among-family variation is additive and would respond to selection. (2) The main effect of increased density is to simply shift the average body size of all families downward (Refstie 1977, Banks 1992, Kavanagh and Olson 2014). (3) Viability selection on body size post-release is strongly non-linear (almost truncational) as has been demonstrated in the Clearwater River (Idaho, USA), Scott Creek (California, USA) and the Cowlitz River (Washington, USA) (Tipping 1997, Reisenbichler *et al.* 2008, Osterback *et al.* 2014).

In this model, under high rearing density a small subset of hatchery families exceed the mean body size at which probability of survival post release is high

(Figure 3.3). These families are the best pre-adapted families to the novel, hatchery environment and likely have traits that are maladaptive in the wild. At high density only the subset of families who are best pre-adapted have high odds of survival and thus, contribute more to the returns of that cohort. In low density, nearly all the families would exceed the mean body size for high probability of survival (Figure 3.3). Under this scenario, the returning cohort would have a larger representation of the families that are maladapted to captivity (the smaller hatchery families at release) and potentially less variance in the number of fish per family at return. The key is that the families that do not perform well in the hatchery are advanced past the threshold for higher survival as juveniles pre-release. Thus, maladapted hatchery families come back in larger numbers to spawn in the wild; carrying with them traits that are likely better adapted to the wild environment. This model has the potential to explain the variance in family sizes at adult return and the tradeoff in performance across hatchery and wild environments (Christie *et al.* 2011, 2014b).

In summary, we do not see strong evidence in support of our original hypothesis that increased density exacerbates the opportunity for among-family selection and causes large changes in rank order performance of families. In light of our findings, we propose an alternate model by which increased density could enhance domestication selection by simply shifting the mean body size of all families downward in the face of strong truncation selection on body size after release from captivity.

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Table 3.1

Intraclass correlations(ICC) and variance components in each tank (by year and density treatment). V_A and V_W are variance components for among and within family, respectively. No significant difference was found between high and low density ICC values in either year.

Year	Density	ICC	V_W	V_A
2012	Low	0.14	49.2	8.0
2012	Low	0.14	50.5	8.4
2012	High	0.17	47.3	9.8
2012	High	0.19	47.9	11.5
2013	Low	0.33	335	165
2013	Low	0.43	334	254
2013	Low	0.35	242	133
2013	High	0.40	190	126
2013	High	0.53	159	180
2013	High	0.37	159	94

Table 3.2

Spearman rank correlation coefficients of family rank order performance for all pairwise comparisons of high and low density tanks.

		2012		
		High 1	High 2	
2012	Low 1	1	1	
	Low 2	0.83	0.83	
		2013		
		High 1	High 2	High 3
2013	Low 1	0.85	0.87	0.94
	Low 2	0.82	0.95	0.95
	Low 3	0.85	0.87	0.94

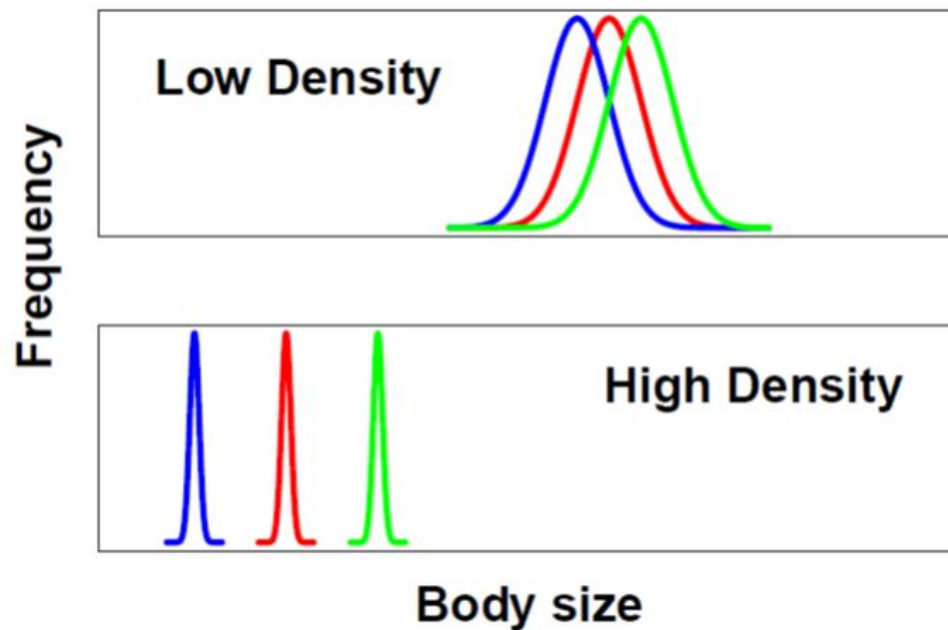


Figure 3.1

Hypothesized distributions of three families (blue, red, green) body sizes in low and high density. In high density larger differences between individual family distributions create a larger opportunity for selection. At low density, family distributions overlap to a greater degree, reducing the among-family variance in performance and opportunity for selection.

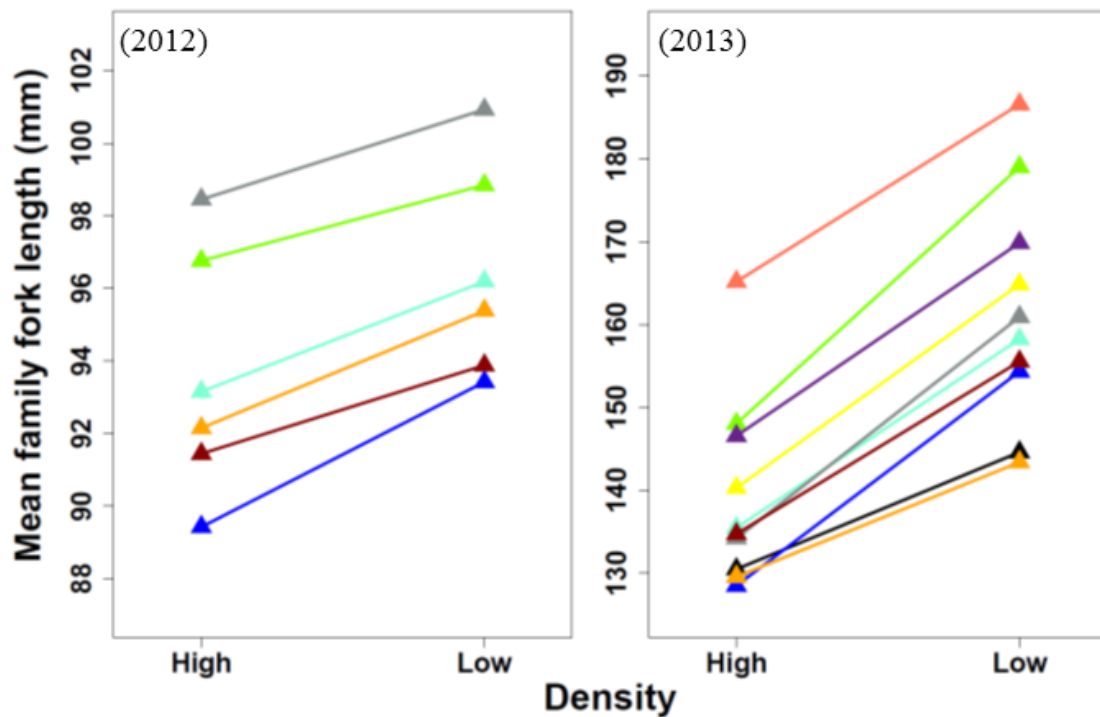


Figure 3.2

Mean family fork length of families used in 2012 and 2013 in high and low density rearing treatments. Each color represents one family. No significant family-by-density interaction was found in 2012 meaning the effect of density did not differ between families. In 2013, a statistically significant family-by-density interaction was found, but explains only 2 percent of the total variance. Thus, the interaction effect is weak compared to the main effects of density and family (which explain 90 percent of the total variance combined).

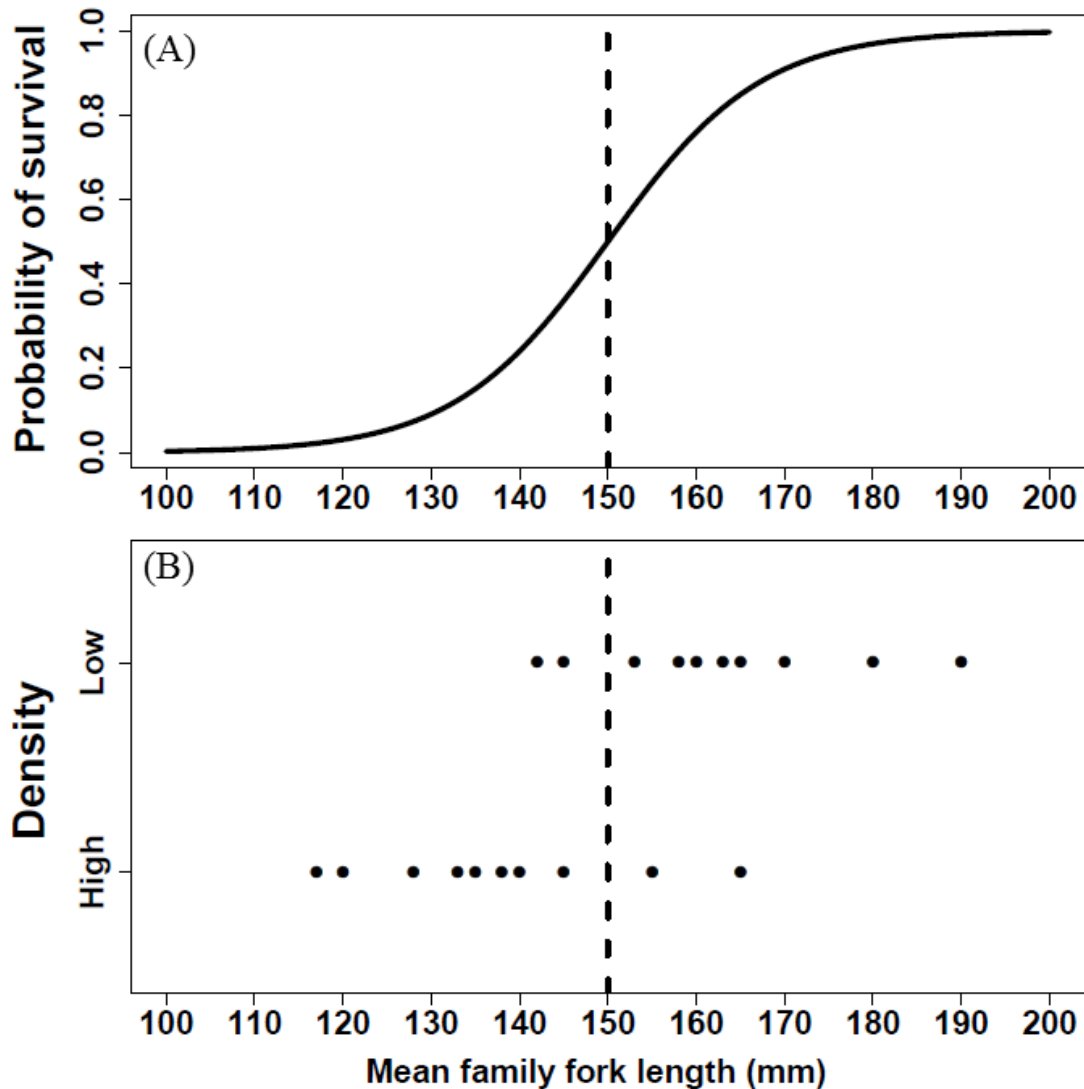


Figure 3.3

Panel A shows a hypothetical survival curve to adulthood with a non-linear shape as is seen in Reisenbichler *et al.* (2008), Tipping *et al.* (1997) and Osterback *et al.* (2014). Panel B shows mean family fork lengths for hypothetical families reared in a hatchery. With truncation selection acting hypothetically at 150 millimeters (denoted by the dashed vertical line); only 2 out of 10 families in high density survive at a high rate and return a large proportion of the hatchery adults. In low density, 8 of 10 families survive at a high rate. This would lead to a more uniform distribution of number of returning adults per family and increase the number of returns for 6 out of the 10 broodstock families substantially. The main effect of density combined with domestication and post-release truncation selection has the potential to explain current patterns of high inter-family variation in adults returns of hatchery steelhead.

CHAPTER 4

The effect of dominance rank on size at release for hatchery reared steelhead (*Oncorhynchus mykiss*)

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Abstract

Salmonid fish raised in hatcheries rapidly adapt to the novel environment. However, what traits are under selection in captivity remains unclear. Hatchery-reared juvenile salmonids have been shown to be more aggressive than wild fish. Therefore, it has been hypothesized that selection in hatcheries favors increased aggressiveness because aggressive fish might dominate food resources and grow faster in captivity. However, whether variation in dominance or aggression correlates with variance in growth rate among-families in hatcheries has never been tested. We tested if average dominance level, determined at ponding (when fish are moved to outdoor rearing), correlates with average body size at smolting (1 year of age) among full-sibling families of hatchery-reared winter steelhead (*Oncorhynchus mykiss*). All families were created using wild parents from the Siletz River, Oregon USA. Fish were raised at either high or low density to see if the correlation was stronger at high density. There was substantial variation among families in average growth rate in the hatchery. However, we found no biologically significant difference in mean body size predicted by rank dominance level, at either density. One explanation may be that increased aggression is simply a correlated response to selection on other traits that enhance growth rate in the hatchery. Or perhaps the aspect of dominance that we measured does not predict growth under the environmental conditions we used. Determining what traits are under selection in hatcheries, and the environmental factors that cause selection, will be key for identifying ways to slow the rate of domestication in hatchery-reared salmonids.

Introduction

Stocking of hatchery reared salmon and steelhead is a widely used conservation tool in the Pacific Northwest to supplement threatened and endangered wild populations (Waples and Drake 2004). One issue with this strategy is that hatchery reared salmon and steelhead often have lower fitness when spawning in the wild than do wild fish (for a review: Christie *et al.* 2014). Adaptation to captivity (via domestication selection) is a known driver of fitness loss in some populations of steelhead (*Oncorhynchus mykiss*) (Christie *et al.* 2011). However, the traits that enhance adaptation to captivity and lead to high fitness before release remain unknown. If the traits under selection are identified then hatcheries may be altered to reduce the effects of adaptation and potentially increase fitness post-release.

What are the phenotypic correlates of variation in family performance in a hatchery? One trait that may be under selection is aggression and dominance. Behavior has a strong effect on the ability of an individual to adapt to captivity (Price 1984) and hatchery reared fish tend to be more aggressive than wild fish as juveniles (for a review: Weber and Fausch 2003). Therefore, higher levels of aggression or dominance may be favored in hatchery culture. Dominant juvenile steelhead obtain more food and monopolize feeding stations compared to sub-dominant individuals (Metcalf 1986, Alanärä and Brännäs 1996). Growth rates of dominant fry (fork length of 30-40 millimeters) are higher than sub-dominant fry over a short time period (approximately one month) (Abbott and Dill 1989), and dominance hierarchies within a class do not change substantially through time, even with changes in body

size (Abbott *et al.* 1985, Alanärä and Brännäs 1996). Salmonids with high dominance rankings have high metabolic rates, making the hypothesis of selection for increased dominance (and high growth that comes from it) particularly compelling (Metcalf *et al.* 1995, Yamamoto *et al.* 1998, McCarthy 2001).

Rearing density may affect the magnitude of the advantage for dominant individuals. For example, hatchery steelhead have been shown to dominate wild fish in high density, but not low density (Riley *et al.* 2009). Additionally, at high density a dominant grouping of fish monopolize food resources, but at low density only the most dominant individuals have been shown to trigger an on-demand feeder (Alanärä and Brännäs 1996). Therefore, if dominance *per se* is under selection, the strength of selection could depend on rearing density.

The fitness of hatchery reared salmonids is correlated with size at release from the hatchery (for a review: Sogard 1997). Larger size at release increases the probability of return for steelhead (Ward *et al.* 1989, Tipping 1997, Reisenbichler *et al.* 2008, Bond *et al.* 2008, Clarke *et al.* 2014, Osterback *et al.* 2014). The families that are best pre-adapted to hatchery rearing are likely those that can grow fast in the novel environment. Steelhead are typically reared in captivity for a single year before release whereas wild fish tend to smolt at 2 years of age or older (Quinn 2005). Thus, selection in the hatchery environment is likely acting on the traits that confer the ability to reach the largest body size possible in a single year. Therefore, it seems plausible that dominance is under selection in the hatchery if it influences size at release.

Using steelhead from the Siletz River (Oregon, USA) we tested for the effects of dominance rank on average juvenile body size at 1 year of age in two density environments. We hypothesized that being more dominant would increase body size in both densities, but dominance level would have a stronger effect in high density. We also tested for the effects of broodstock (parents) body size and ponding length on body size to determine if those traits predicted size at release. Positive effects of broodstock size on juvenile size have been found previously (Gjedrem 1983, Gjerde and Schaeffer 1989, Hu *et al.* 2013), but we wanted to determine if they affected juvenile body size to a similar degree as dominance level.

Materials and Methods

Sample collection and spawning procedures

Adult winter steelhead were collected at a fish trap in the Siletz River (Oregon, USA) by Oregon Department of Fish and Wildlife (ODFW). Broodstock were transported to the Alsea River hatchery and spawned following standard ODFW protocols (Oregon Department of Fish and Wildlife 2013). We created 7 families using 1 male and 1 female with no broodstock individual being used in more than one cross. All broodstock were of wild origin as determined by the presence of the adipose fin. For each broodstock we measured fork length and took a tissue sample (stored in 95% ethanol) for parentage analysis.

After fertilization and water hardening, embryos were transported to the Oregon Hatchery Research Center (OHRC) in Alsea, Oregon. All embryos were kept

in separate family groups until approximately two weeks after first exogenous feeding.

Density treatments for growth rate

Fry were randomly chosen from each family and put into one of two treatments. The high density treatment (140 fish/m³) was set up in 1.8 meter diameter tanks while the low density treatment (20 fish/m³) was set up in 3.6 meter diameter tanks. All tanks were tan fiberglass with netting covering the surface to provide shade, cover and protection from avian predation.

Three replicates of each density treatment were created on July 15, 2013. At the time of treatment application, juveniles were moved from the indoor incubation room to the outdoor tank farm. All individuals had the adipose fin removed during January 2014 following ODFW procedures to mimic the production process as closely as possible (Oregon Department of Fish and Wildlife 2013)

Fish husbandry

All treatments were fed to satiation daily with Bio-Oregon commercial fish feed. This included 6-8 feedings per day until fish reached 0.75 grams; 4-6 feedings until the fish were 3 grams then 2-4 feedings per day for the remainder of the experiment (R. Couture, Oregon Department of Fish and Wildlife, personal communication). Rearing procedures including tank cleaning and health monitoring followed the ODFW Alsea hatchery operations plan for Siletz stock winter steelhead (Oregon Department of Fish and Wildlife 2013).

Growth rate sampling

After 9 months of outdoor rearing all fish were euthanized via overdose of MS-222 following procedures outlined by the American Veterinary Medical Association (Leary *et al.* 2013). Fork length was measured in millimeters for each individual and a fin clip was taken for genetic parentage analysis. Fin clips were stored in 95% ethanol.

Genetic parentage analysis

Genotypes at 6 microsatellite loci were used to assign juveniles back to family groups. DNA was extracted using Chelex 100 following protocols of Nelson *et al.* (1998). The SPAN B suite of loci (*Ogo4*, *Omm1046*, *Omy7*, *One102*, *Ots4*, *Ssa407MP*) were amplified using the following PCR thermal cycling regime: 95 °C for 15 minutes, 35 cycles of 94 °C for 30 seconds, 57 °C for 90 seconds, 72 °C for 60 seconds with a final extension of 60 °C for 30 minutes (Stephenson *et al.* 2009). All loci were multiplexed in a single reaction for each fish. Genotyping was performed on an ABI 3730 capillary electrophoresis system (Applied Biosystems, Foster City, California) at the Oregon State University Center for Genome Research and Biocomputing. GeneMapper version 4.1 (Applied Biosystems, Foster City, California) was used to score the genotype data.

To perform parentage analysis we used the SOLOMON program with simple exclusion because broodstock pairings were known (Christie *et al.* 2013). Juveniles who mismatched at any loci were checked manually to determine the putative broodstock pairing to which they belonged.

Dominance behavior experiment

The dominance trials were conducted on siblings of the fish raised for the growth rate measurements. Trials were started the same time the fish in the growth experiment were moved outdoors. The mean dominance rank of each family was calculated in both low and high density treatments. Six indoor flumes contained a total of 32 rearing baskets, alternating large (58_x33_x18 cm L_xW_xH) and small (33_x13_x20 cm L_xW_xH) size to create different density treatments (Figure 4.1). One fish from each family was placed in each rearing basket (7 fish total in each basket). To distinguish individuals from each family a fluorescent visual implant elastomer tag (Northwest Marine Technology) was injected on both sides of the dorsal fin to produce a unique color combination. The color combination was randomly assigned to each family within a rearing basket to avoid any potential color bias associated with dominance. Each fish was anesthetized (to a state of lost equilibrium) with MS-222 prior to tagging. After tagging, the fish were placed in a rearing basket and allowed to recover for 24 hours before the experiment commenced.

Each basket was fed 4 times per day at the upstream end of the basket. A small amount of food was administered at each feeding session creating competition for food within the baskets. The fish that held the most upstream position (closest to food application point) was deemed the dominant fish. This methodology has been used by numerous researchers to evaluate dominance of juvenile salmonids (Berejikian *et al.* 2000, Riley *et al.* 2009).

Juveniles competed for 24 hours at which point the dominant fish in each basket was identified. Identification was performed in the dark with an ultraviolet flashlight that fluoresced the elastomer tags. This was the least invasive method to identify which individual held the most upstream position. Each basket was observed for approximately 2 minutes to confirm that the individual at the most upstream point was the dominant fish. After confirming the fish at the upstream-most point was dominant (did not leave the position and attacked others who attempted to invade the area) a small aquarium net was used to remove the fish. Immediately after removing the fish it was euthanized with an overdose of MS-222, measured for fork length (mm) and assigned a dominance rank according to the day it was removed (day 1 corresponded to a rank of 1). After all dominant fish were removed for the day all rearing baskets were fed. The experiment lasted six days until two fish remained in each basket.

The numerical ranking system had no quantitative value and was used as an ordered factor in the statistical analysis. Because two families had the same mean dominance rank we assigned them both the same dominance rank and then skipped the next rank value. For example, we assigned two families a dominance rank of 2 and then assigned the next most-dominant family a rank of 4. In the data analysis we compared dominance level to mean body size within a density (i.e. low density dominance level with low density growth).

Statistical analysis

To determine if dominance rank correlated with juvenile body size we used linear mixed effects models. Mixed effects model were used to account for variation and correlation within and among tanks. We tested if an ordinary least squares regression fit the model to an equal degree as the mixed model via a likelihood ratio test (Zuur *et al.* 2009). If a mixed model was preferred we evaluated the fixed effects using maximum likelihood (ML), but reported the final model after re-fitting with restricted maximum likelihood (REML) following Zuur *et al.* (2009). All analyses were performed using R version 2.15.1 (R Core Team 2012). The nlme package was used for fitting all mixed effects models and producing confidence intervals (Pinheiro *et al.* 2012).

In order to satisfy the assumptions of the mixed modeling analysis we transformed the response variable (fork length) with the following Box-Cox transformation: $y' = (y^\lambda - 1) / \lambda$ where y is the fork length of an individual and $\lambda = 2$. The following two full models were analyzed:

$$\text{Eq 4.1 } y' = \beta_0 + \beta_1 (\text{Density}) + \beta_2 (\text{Dominance Rank}) + \beta_3 (\text{Density*Dominance Rank}) + \alpha + \varepsilon$$

$$\begin{aligned} \text{Eq 4.2 } y' = & \beta_0 + \beta_1 (\text{Density}) + \beta_2 \times (\text{Mother Fork Length}) + \beta_3 (\text{Father Fork Length}) \\ & + \beta_4 (\text{Ponding Length}) + \beta_5 \times (\text{Density*Mother Fork Length}) + \\ & \beta_6 \times (\text{Density*Father Fork Length}) + \beta_7 (\text{Density*Ponding Length}) + \alpha + \varepsilon \end{aligned}$$

where y' represents the Box-Cox transformed mean family fork length, α is a random term for tank and ε represents a random error term. Interactions with density were included to account for differences in fixed effects based on the environment the fish were reared in.

Results

A mixed model with tank as a random factor was preferred over a fixed effects model (Likelihood ratio test, $p < 0.0001$). A significant interaction between density and dominance rank was present (linear mixed effects model, $p < 0.0001$). Significant main effects of density and dominance rank were also present (linear mixed effects model, $p = 0.005$ and $p < 0.0001$, respectively). Although the interaction and main effect of dominance rank were statistically significant they explain 30% of the total variation (15% due to dominance rank and 15% due to the interaction). The main effect of density accounted for 58 % of the total variation. The effect of dominance rank and the interaction between density and dominance rank on juvenile fork length do not appear biologically significant (Figure 4.2). The mean fork length of each dominance rank is within approximately one centimeter of each other which we argue is not a biologically significant difference (Figure 4.2).

The mixed model analyzing equation 4.2 was not preferred statistically over the fixed effects model (Likelihood ratio test, $p = 0.25$), but because of the correlation of observations within a tank we chose to proceed with the mixed model. This is conservative, but accounts for within tank correlations. No interactions between density and ponding length, density and father fork length or density and mother fork

length were present (linear mixed effects model, $p = 0.94$, $p = 0.99$, $p = 0.84$ respectively). Main effects of ponding length, father fork length and mother fork length did not influence fork length (linear mixed effects model, $p = 0.19$, $p = 0.35$, $p = 0.94$ respectively). Density did significantly affect juvenile body size (linear mixed effects model, $p = 0.005$) with low density fish being 2.2 cm larger (95% CI 1.1, 3.4 cm) than fish reared in high density.

Discussion

We did not find support for the hypothesis that high levels of dominance correlate with large body size in hatchery reared steelhead. The only effect that was significant in predicting body size was rearing density, which was expected. The size of the broodstock had no effect on size at release. The length of a family at the time of ponding, when fish are moved from indoor rearing to outdoor tanks also had no effect on final body size. Although there were differences in family body size (Figure 4.3), no phenotypic trait we were able to measure correlated with body size at the end of one year.

There are multiple potential explanations for not finding support for our original hypothesis. First, there could be low statistical power given only six dominance ranks. On the other hand, there was no trend in the data suggesting even a weak positive correlation. Second, the type of dominance that we measured (ability to defend a predictable feeding site) may not predict enhanced growth rate in the hatchery environment experienced by our fish. For example, because feed is thrown on the surface of the tanks, perhaps ability to maintain a position near the surface of

the water is the trait actually under selection. Indeed, domesticated masu salmon (*O. masou*) spend more time near the surface in a hatchery environment than do wild masu (Reinhardt 2001).

Alternately, dominance *per se* may not affect growth rate, but increased dominance may be simply a correlated response to selection on other traits that actually influence growth rate. One plausible trait under selection is metabolic rate. Dominant individuals have higher metabolic rates (Metcalf *et al.* 1995, Yamamoto *et al.* 1998, McCarthy 2001), allowing them to process food faster and potentially turn more energy into growth. Second, dominant individuals may use less energy for maintenance functions or locomotion and therefore have more energy for growth (Ejike and Schreck 1980, Metcalfe 1986).

In conclusion, we found no phenotypic or dominance trait that we could measure to predict fork length at release. The hypothesis for high level dominance correlating with high levels of growth in the hatchery was plausible from previous findings, but the novel (high food, predator free) environment may not be favoring dominant individuals *per se*. We hypothesize the traits that underlie a dominant phenotype may be what are favored in captivity and not the dominant phenotype itself. High metabolic rates, large proportions of energy for growth and minimal energy expenditure for maintenance may be what are driving high growth rates in the hatchery.

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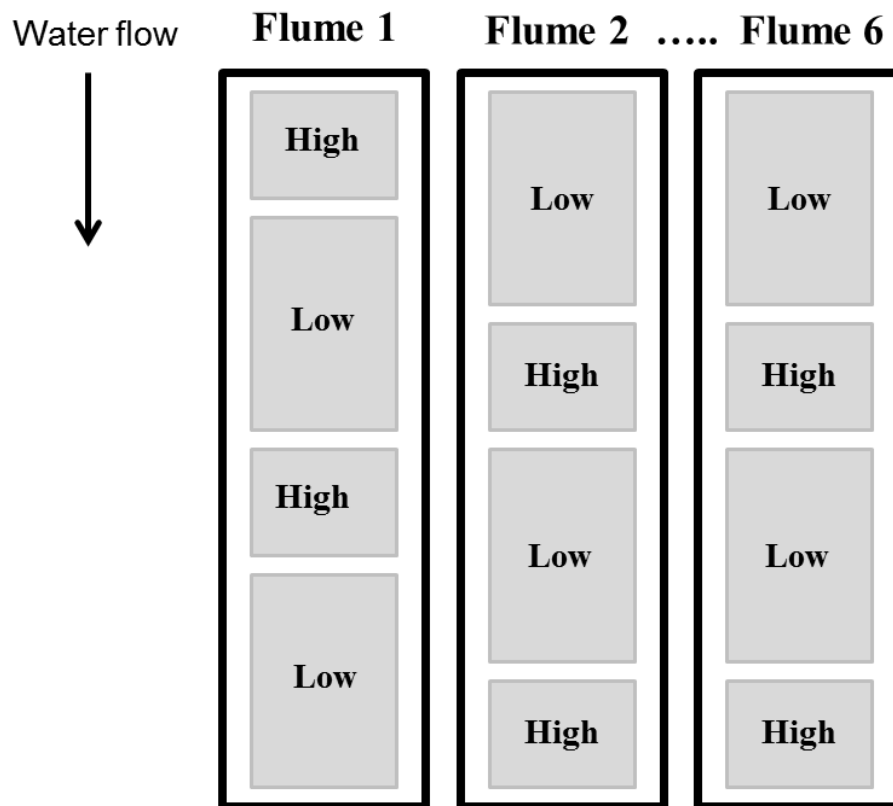


Figure 4.1

Dominance behavior experiment. Six flumes were used to house 32 individual rearing baskets. Large and small rearing baskets (low and high density treatments) were alternated within a flume. Each basket was fed at the upstream end to establish a dominance hierarchy. The fish that was closest to the head of the basket was determined to be the dominant fish.

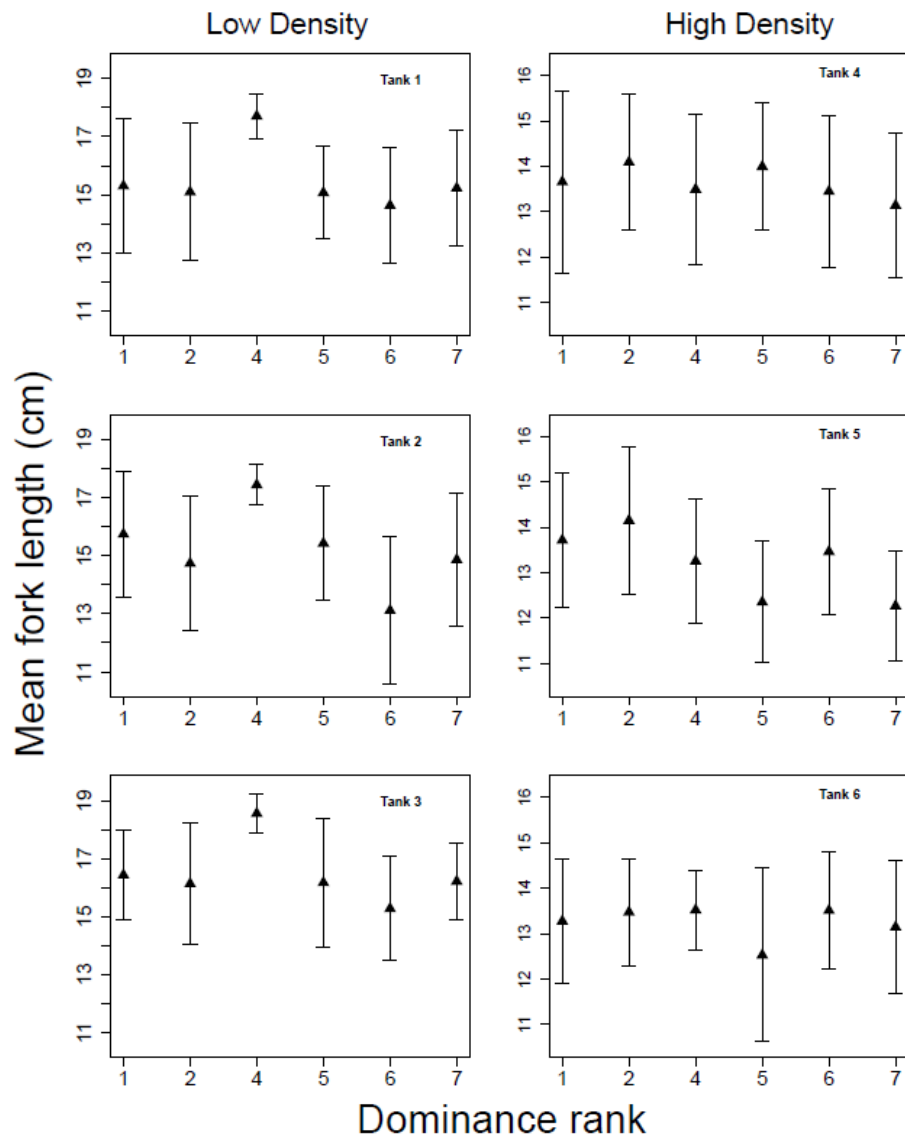


Figure 4.2

Mean fork length (± 1 SD) plotted against dominance rank within each tank. Dominance rank was statistically significant, but we see no trend that is biologically significant. All dominance ranks are approximately within 1 cm of each other in mean fork length that we argue is not a biologically significant difference at this lifestage.

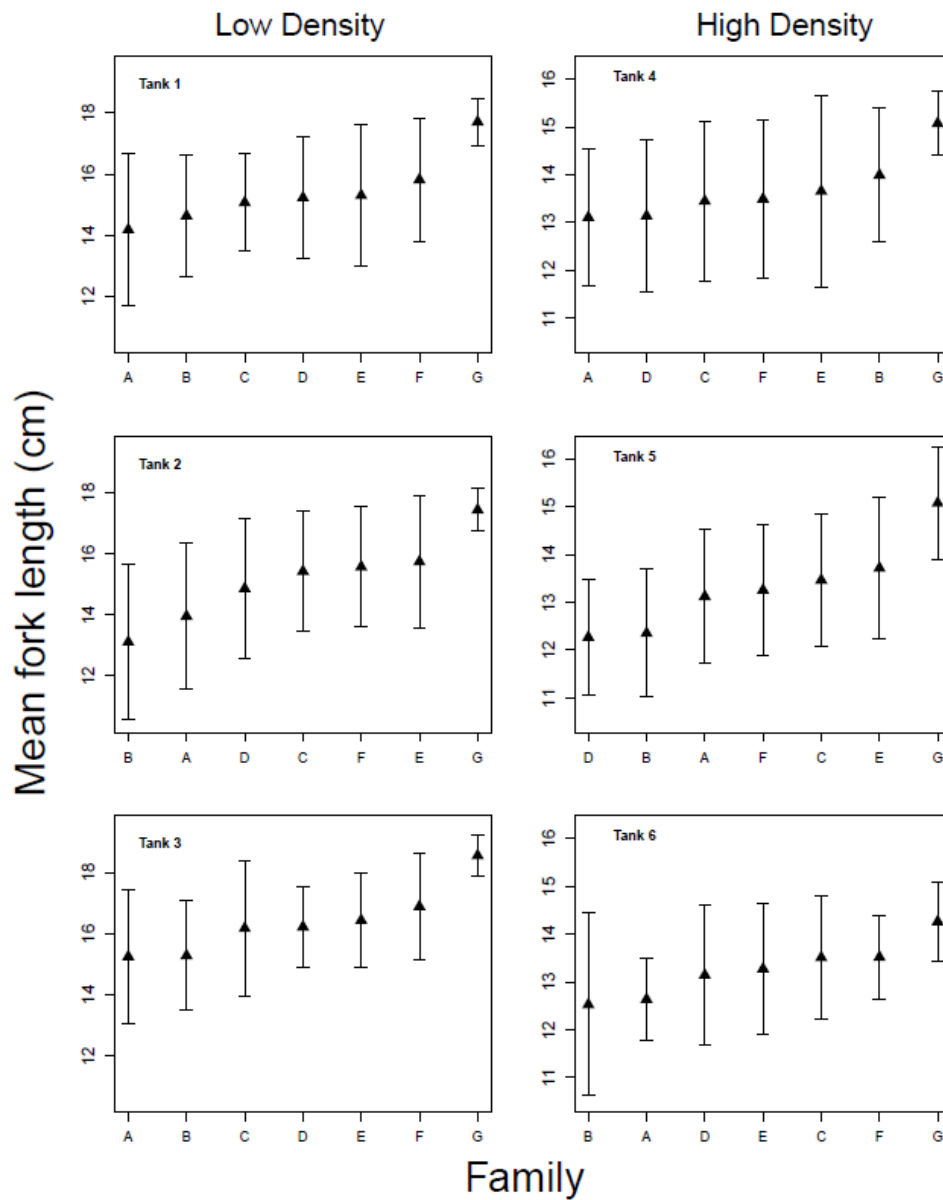


Figure 4.3

Mean family fork length in low and high density rearing treatments. Differences between families exist, but none of the traits we measured correlated with these differences.

CHAPTER 5
CONCLUSION

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Supplementing salmon populations with hatchery reared fish for restoration and conservation is a highly controversial issue. The crux of the debate is that (1) captively reared fish have lower fitness than wild fish when spawning in the wild and (2) hatchery fish may be having negative impacts on the ability for a population to increase in size. One solution is to identify the mechanisms and traits influencing fitness loss in hatchery culture and determine if they can be reduced or eliminated to produce hatchery fish with equivalent fitness to wild fish. My dissertation was aimed at identifying a possible driver of adaptation to captivity and the traits that are under selection in the hatchery that lead to fitness loss in the wild.

One trait that may be under strong selection in the hatchery environment is growth rate. Body size at release is correlated with probability of survival for steelhead and salmon. Thus, the families that are able to grow fast in a single year in the hatchery (versus 2 or more years in the wild) may be selected for in hatchery conditions. In my growth rate experiments I did not find a difference in body size between first generation hatchery and wild fish reared in a common environment. I did find positive effects of mother length on juvenile body size and an environmental effect to influence body size in the hatchery. The date a family was spawned was correlated with body size with earlier spawned families being larger. A combination of environmental and genetic effects contributes to growth rate in captivity, but no difference between hatchery and wild fish was present. One potential explanation for not finding a difference between hatchery and wild fish is that the experimental rearing conditions did not identically replicate the production level rearing conditions.

The experiments I conducted were designed to match the production process as closely as possible, but differences in rearing density may have not made the experimental environment stressful enough for growth rate differences to manifest themselves.

The strength of a performance tradeoff in Hood River steelhead between hatchery and wild environments was found to correlate with rearing density that the hatchery experienced during captivity. Thus, rearing density may be a mechanism by which domestication selection is acting during captivity. In my experiment examining the effect of rearing density on the opportunity for selection, I found high densities did not increase the total amount of variation between families in body size. Because no difference in variation was found the opportunity for domestication was not influenced by rearing density. Rank order performance of each family was highly correlated between high and low density suggesting that families who are best pre-adapted to hatchery rearing will have relatively high performance regardless of the density they are reared in. This finding led to a novel hypothesis of how domestication during captivity and selection after release could drive differences in fitness in the wild. Under typical high rearing conditions only a small number of families attain a body size above a threshold for high survival after release. Hatchery returns of adults are dominated by a few families that are likely the families that have the largest body sizes at release from the hatchery (i.e. the best pre-adapted families to captivity). The families that are best adapted to hatchery rearing likely have traits that are maladaptive for high fitness in the wild. Highly pre-adapted families return

in large numbers compared to poorly adapted hatchery families and thus, because more highly adapted hatchery individuals are on the spawning grounds, they may be driving the difference in fitness. If the captive environment can be altered for maladapted hatchery families (those with small body sizes at release) to grow big enough and surpass the threshold for high survival post-release, then differences in fitness in the wild environment may be reduced.

My fourth chapter sought to determine if high levels of dominance correlated with large body size at release for hatchery steelhead. Dominance is a trait that could be under selection in captivity because hatchery fish are known to be more aggressive than wild fish as juveniles and dominant fish have been shown to grow faster over a short time period (approx. 1 month). I did not find a biologically significant effect of dominance on fork length after a year of captive rearing. One potential explanation for not finding an effect of dominance is that behavior is not being selected for, but the physiology that drives differences in behavior is truly under selection. High metabolism correlates with dominance level and perhaps metabolic rate is being selected for with dominance being a correlated response.

The goal of my dissertation has been to identify what mechanisms are driving fitness loss in hatchery reared steelhead and what specific traits are under selection in captivity. Although I did not find a specific trait to be under positive selection in the hatchery or a mechanism that enhances the opportunity for selection to act, I have advanced the understanding of the dynamics involved in hatchery rearing that influence family performance. I've advanced toward my dissertation goal by

identifying that dominance level does not correlate with fitness in captivity, rearing density has very little effect on the ability for selection to act and differences in growth rate are not apparent between wild and first generation hatchery fish. In the future, if hatchery fish continue to be used as a conservation tool the forces driving adaptation to captivity and the traits under selection need to be identified. By doing this we can potentially change the captive rearing environment so fitness differences can be reduced or eliminated. To borrow a line from Elmer Crow of the Nez Perce Tribe – “how can you let a species that’s been around for millions of years go extinct in only a few hundred years?” I think Elmer said it best, and we have a responsibility to identify and remedy what is causing fitness loss in hatcheries if we are going to continue to use hatchery reared fish as a conservation tool to restore populations of salmon and steelhead.

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