EFFECTS OF DOMESTICATION ON BEHAVIOR IN CLONAL LINES OF HATCHERY-REARED RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

BY

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of Sharon M. VillageCenter find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

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There are currently nine clonal lines of rainbow trout propagated in the Thorgaard lab. This experiment was designed to determine if these clonal lines, which have varying degrees of domestication, differ in their behavior. The hypothesis was that behavioral differences among the lines are related to the level of domestication. The samples used for this study included clonal lines created from fish whose progenitors had been in a hatchery setting for varying lengths of time (more than 20 generations, 2-20 generations and zero generations (produced from gametes collected directly from wild fish)). The behavioral assays included mean swim level, activity level, feeding latency, feeding frequency, type of startle response and post-startle mean swim and activity levels. All assays showed significant differences between strains, though not all strains showed the patterns we would have expected due to their level of domestication, nor were all strains consistent across all behaviors. Thus this study shows that it is not possible to predict behavioral patterns based on domestication history alone.

Keywords: behavior, clonal lines, domestication, rainbow trout, *Oncorhynchus mykiss*

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Dedication

This thesis is dedicated to my mother.

CHAPTER ONE

INTRODUCTION

1. Effects of domestication on behavior

Domestication is the process in which animals become accustomed to the presence of humans and become dependent on humans for survival. Normally, animals are very timid and uninterested in or avoid human contact. When they become domesticated their phenotype changes. These phenotypic changes have been observed in many different species of animals.

Domestication has been most extensively studied in mammalian species. For example, silver foxes (Belyaev, 1969, Spady and Ostrander, 2007), wolves (Saetre et al., 2004) and mice (McPhee, 2004) have all been studied for changes involved in the domestication process that include behavioral and phenotypic changes as well as changes in gene expression. Domestication generally has similar behavioral effects for mammalian species such as tameness which causes them to readily approach humans and tolerate, even apparently enjoy, human contact (Belyaev, 1969, McPhee, 2004).

One of the best known studies involved silver foxes, *Vulpes vulpes* (Belyaev, 1969). This study involved foxes that were produced at fur farms in the Soviet Union. In this study, silver foxes were selectively bred to produce a phenotype that was more similar to the domestic dog. The foxes became interested in human contact and even started exhibiting dog-like behavior such as barking, tail wagging and whimpering. They also showed changes in coat color and slight morphological changes in body dimensions.

However, domestication in fishes is slightly different. Due to many anthropogenic factors, it has been necessary to establish hatcheries for salmonids in order to maintain, or

attempt to increase, wild populations. Traditional hatcheries were focused mainly on quantity of fish released. This caused unintended phenotypic changes. Many of these changes involved a lack of selection pressures leading to a number of traits that were undesirable for the natural habitat. Less fit individuals were created and allowed to reproduce in the hatchery environment which, in the long run, altered the gene pool in a negative way if they were eventually to be released. However, the changes were allowed to occur because they were ideal for the hatchery setting. The more domesticated the fish became the better the profits for the commercial hatcheries. The fish in commercial hatcheries generally exhibit higher growth rate which is beneficial for hatcheries that do not release fish into the wild but instead process them as soon as they reach marketable size. This allows them to get fish to the market faster, which not only provides profits sooner, but also decreases the cost to maintain the hatchery population.

Domestication in fish generally involves physiological changes, such as increased growth and development rate (Huntington, 2004, Petersson et al. 1996, Tymchuck et al. 2006), as well as changes in behaviors. However, it is not always apparent how behavior changes. Some behavioral changes that tend to lead to decreased fitness include aggression, decreased magnitude or duration of anti-predator responses, higher mean swim level and changes in reproductive behavior.

In some behavioral traits, aggression and activity level for example, the intensity level, or lack thereof, are inconsistent with domestication history and may depend on species or strain. Fish can become more relaxed in some of their behaviors while other behaviors become magnified. Two examples of behaviors that generally become more relaxed as domestication level increases are the anti-predator response (either ceasing all

movement when disturbed or entering a shelter), and mean swim level in the water-column which is defined as the mean swimming level relative to the surface or the substrate. The mean swim level of domesticated fish tends to be higher, thus making them more susceptible to surface predation (Lucas et al. 2004, present study) while the anti-predator response generally decreases in some way (Alvarez and Nicieza, 2003, Berejikian 1995, Huntington, 2004, Johnsson et al. 1996, Lucas et al. 2004, Malavasi et al. 2008, Naish et al. 2008, Yamamoto and Reinhardt, 2003). Examples of behaviors that can either magnify or decrease upon domestication include aggression and activity level. Hatchery-reared fish have been shown to be more aggressive than their wild counterparts (Kelley et al. 2006) in some cases, but less aggressive in others (Berejikian et al. 1995, Berejikian et al. 2001b). These studies suggest many factors are involved in aggressive behavior, such as size of fish, prior residence and domestication history. Changes in activity level vary and it is hard to predict how it will differ among strains of domesticated and wild fish (Robison and Rowland, 2005). Domestication has also led to morphological changes in some species of fish (Hard et al. 2000, Wright et al. 2006); whether these changes are detrimental to survival and reproductive fitness has yet to be determined. Clearly, domestication results in many phenotypic changes compared to the wild counterpart. These behavioral, physiological or morphological changes are generally counterproductive when these animals are released back into the wild.

Some avenues to address the problematic issue of domestication in brood stocks used for supplementing natural populations include quantitative trait loci (QTL) and gene mapping studies to locate genes responsible for detrimental or beneficial behaviors, microarrays to look at gene expression which would offer insight into the genes that are

being expressed in particular individuals or clonal lines, as well as further behavioral studies looking at additional behaviors. Behavioral QTLs have been found in a variety of different species such as junglefowl and White leghorn layers (Jensen and Andersson, 2005), zebrafish (Wright et al. 2006), and rainbow trout (Drew et al. 2007). Microarrays have been used to look at gene expression in wolves and dogs (Saetre et al. 2004), which are extremely similar genetically, and differences have been discovered. Microarrays are becoming more widely used and have the potential to offer much more insight into domestication and hatchery environments.

Some recent work shows hatchery-reared fish can be equally successful as their wild counterparts (Araki et al. 2006, Berejikian et al. 2008) when the stock is taken from the area it is intended to supplement. In the Araki et al. (2006) study, fish were taken from the wild at the eyed stage and reared in either earthen ponds or circular vessels. The authors measured the number of redds produced by hatchery-reared fish compared with wild fish and found no differences. Also, there is some evidence from different studies that innate abilities can be retained despite being in the hatchery environment for generations. The innate abilities retained were nesting ability (Scott et al. 2005) and the fright (freeze or flight) response (Scheurer et al., 2007).

Although domestication can have detrimental effects on survival of fish released back into the wild, studies have shown effects of domestication can be limited by supplementing hatchery stocks with wild stock endemic to the area of intended release (Araki et al., 2006, Berejikian et al., 2008, Scott et al. 2005, Scheurer et al., 2007). When this method is used, the fish that are released have performed much better than the traditional hatchery stock in choosing nesting sites, preparing redds and showing

courtship behaviors. Placing fish in a more natural environment such as an earthen pond or simulated stream has been shown to increase fitness (Scott et al. 2005, Scheurer et al. 2007). Hopefully, hatchery practices will begin to implement some of the methods described above and the fish used to supplement the wild stocks will begin to enhance the wild populations.

CHAPTER TWO

EXPERIMENTAL DATA AND DISCUSSION

1. Introduction

Due to many anthropogenic factors, such as dams and pollution, salmonid populations in the Pacific Northwest continue to remain much lower than historical numbers. Low population sizes have made it necessary to supplement various populations of salmonids with fish reared in hatcheries. Hatcheries are now common fixtures in the Pacific Northwest, as well as many other places in the world.

One of the problems associated with hatcheries is the potential for fish to become domesticated. Domestication is the process that causes physiological, morphological, behavioral and genetic changes resulting in a phenotype that is adapted to and dependant on human contact. It has been shown many times that domesticated animals exhibit behaviors that differ from their wild counterparts (Araki et al., 2007, Belyaev, 1969, Berejikian et al., 2004, Berejikian, 1995, Lucas et al., 2004, Malavasi et al., 2008, Moretz et al., 2007, Petersson et al., 1996, Saetre et al., 2004, Yamamoto and Reinhardt, 2003). Some of these differences are attributed to stocking densities, methods in which food is provided and lack of predation pressure. Domesticated fish generally feed more actively and position themselves higher in the water column than do wild fish (Lucas et al. 2004). This is possibly due to the high stocking densities, which create higher levels of competition for food, as well as food delivery methods, which are generally at or above the surface. Also, there are many commercial hatcheries that are interested in filling the food-fish gap left by dwindling wild fish populations. These hatcheries often select for fast growth in order to obtain market size and high market value in the shortest time

possible. The lack of natural selection such as mortality by predation in these facilities leads to drastic changes in genotype and phenotype (Araki et al. 2008, Naish et al. 2008). While these hatcheries are not necessarily a problem themselves, problems arise when the same methods are applied to both commercial and supplementation hatcheries. The fish that are produced under these conditions are not as fit for survival or reproduction in a natural environment as wild fish are. They generally have a lowered anti-predator response (Johnsson et al. 1996, Malavasi et al. 2008, Scheurer et al. 2007, Yamamoto and Reinhardt, 2003) which could cause higher mortality by predation. When the survivors return to spawn they have difficulty establishing dominance over the wild fish (Metcalfe et al. 2003, Yamamoto and Reinhardt, 2003), which could cause them to spawn in less than ideal spawning sites, thus reducing reproductive success, another fitness component. The behavioral changes that occur as a result of domestication are the focus of this study.

This study was modeled after a previous experiment (Lucas et al. 2004) which also studied behavior in some of the clonal lines being reared at Washington State University in the Thorgaard lab. The previous study, however, looked at behaviors in only four of the clonal lines and utilized hybrids of the four clonal lines with outbred females. This project involved eight of the nine currently available clonal lines, utilized pure clonal fish, and also included additional behavioral measures. The fish in this study were androgenetic clones produced via methods presented in Parsons and Thorgaard (1985).

The purpose of this study was to establish a baseline of behaviors for each of the clonal lines in our lab. Unfortunately, one line—Skookumchuck—did not produce sperm at any time during the study and therefore could not be used.

This study included eight clonal lines of rainbow trout with varying degrees of domestication. Since experiments depended on fish availability and it was not possible to look at all nine lines simultaneously, this study is composed of three separate experiments, each using the same methods and measuring the same set of behavioral traits, but conducted on different clonal lines at different time periods. The first experiment was conducted in May of 2007 and included the Arlee, OSU, Skamania, Klamath and Whale Rock clonal lines. The second experiment was conducted in June of 2007 and included the Arlee and Swanson clonal lines. The third experiment was conducted in March of 2008 and included the Arlee, Hot Creek and Clearwater clonal lines. Because Arlee is one of the most highly domesticated lines currently in propagation, the Arlee males spawn through much of the year and were used in all three experiments. It was hoped that the Arlee line could be used as a control, with the prediction that it would show no differences in behavior across experiments because it was a clone, but that was not the case. Because the Arlee line showed significant differences from experiment to experiment, it was necessary to analyze each experiment separately. In an effort to more easily visualize the results using the various lines, the data was normalized to the Arlee results in each experiment and presented on one graph.

The behavioral assays were designed to measure some of the changes in behavior known to occur as fish become accustomed to the hatchery environment. The behaviors of interest included activity level, feeding latency and number of feeding attempts, mean swim level, startle response, post-startle mean swim level and post-startle activity level (Lucas et al. 2004, Robison et al. 2005). A primary purpose of this study was to determine if differences in the behavior of the clonal lines were consistent with the duration of their

domestication (i.e. number of generations). We examined the clones for patterns expected to exist in domesticated lines. It has been shown that more domesticated fish tend to swim higher in the water column, have increased foraging behavior and differ in the type of their startle response (Lucas et al. 2004), a measure of their response to presence of a predator and thus a potential predictor of their survivability.

Genetic analysis of behavioral differences detected between clones using QTLs could be an important future research direction. This would involve crossing clones with different behaviors, then producing progeny from the hybrid which segregate for numerous DNA markers and the trait(s) of interest.

2. Materials and Methods

2.1 Clonal lines

Clones were created using protocols set forth in Parsons and Thorgaard (1985) and Scheerer et al. (1991). The fish used in this study represent all but one of the clonal lines currently in existence in the Thorgaard lab. Each of the lines represents a specific source population and their domestication histories are known. The clones represent varying degrees of domestication and are loosely grouped into three classifications: highly domesticated, semi-wild and wild. The highly domesticated lines include Arlee, Hot Creek and OSU. These lines have been propagated in the hatchery setting for approximately 100 years (Robison et al. 1999) and all originate from the same general area, the Upper Sacramento River drainage, specifically the McCloud River (MacCrimmon 1971, Needham and Behnke, 1962). The lines that are considered semi-wild include Swanson, Skamania, Clearwater and Klamath. They are considered semi-wild because they are either recently derived from wild stock, supplemented with wild

stocks periodically or they are steelhead lines. The steelhead lines are released and spend 2-3 years in the ocean and thus are considered more wild than the highly domesticated fish due to the selection pressured they encounter in the ocean and on their migration route. The line that represents the wild type is the Whale Rock line. It was cloned from gametes taken directly out of the wild on a reservoir tributary in central California.

2.2 Experimental design

Clone production: Experimental fish were produced using irradiated outbred eggs fertilized with sperm from the desired clonal line and heat-shocked to induce diploidy. The details of this method are described in Parsons and Thorgaard (1985) and Young et al. (1998).

Fish rearing: The fertilized eggs were placed in incubators to grow to the desired size, which was the 'swim up'stage when they would absorb their yolk sac and begin feeding normally. At swim up, each fish was transferred to its own individual tank and left overnight to acclimate in a 14ºC cold room, where the observations occurred.

The tank in which each individual fish was behaviorally studied had three sides painted with white paint and only the front of the tank was clear. Total tank volume was seven liters and tanks measured 26cm long, 16cm wide and 17 cm high. However, the experimental tanks only held five liters of water that consisted of four horizontal levels of three cm each and two vertical levels which represented each half of the tank. The tanks were set up in a stand consisting of 18 cubicles (units), with each unit measuring 47cm wide and 50cm high. They were arranged three units high and six units long. There was a blind attached to the front of the cubicles that extended approximately 45cm from the front of the tanks to the observer. Each cubicle was individually lit and shielded from any

additional light by the blind and the curtain in the back of the tank. The lights were automatically controlled with lights on from 0500 to 2100.

Behavioral measurements began the morning after a fish was transferred to its individual tank. Behaviors were recorded at three times during the day for three days for each fish. The first measurements were taken between 0730 and 0830, the second between 1230 and 1330, the third between 1630 and 1730. The behaviors recorded in the morning were activity level, mean swim level, feeding latency and number of strikes. At mid-day, activity level, mean swim level, startle response, post-startle activity level and post-startle mean swim level were recorded. In the evening only activity level and mean swim level were recorded. After three days the fish were euthanized and tissues were preserved for future use.

Because the clones were not available simultaneously, behaviors were recorded in three separate experiments, May 2007, June 2007 and March 2008. Each of the experiments followed the same methods but contained different clonal lines. Experiment one used Arlee, OSU, Skamania, Klamath and Whale Rock. Experiment two used Arlee and Swanson. Experiment three used Arlee, Clearwater and Hot Creek.

2.3 Behavioral Assays

Behavior was observed as it occurred, rather than through the analysis of videotaped recordings.

Mean swim level (MSL), or use of the water column, was scored as the mean of the four horizontal levels that the fish occupied for 5 seconds, each second one level was recorded as being occupied. Each tank had four levels of 3cm each and the levels were scored as the number above the floor of the tank, with the floor of the tank being zero. If

the fish was in the water column between the floor and the first horizontal first line, it was scored as one. Therefore, fish MSLs were scored as one, two, three or four and the average of the levels from the five second count was the final MSL.

Activity level was determined by a visual scan lasting one minute. The activity level was scored as the number of cell changes the fish made during that minute in relation to the 4 X 2 grid drawn on the front of the tank. The grid consisted of the 4 horizontal levels with a line drawn vertically down the center of the tank to add an additional two vertical levels.

Feeding latency was determined as the number of seconds it took the fish to attempt to feed after the food was introduced. Food was delivered in pre-measured 0.1g amounts, manually, through the opening in the lid of the tank.

Feeding attempts, or strikes, were calculated as the number of feeding attempts, either successful or not, during one minute after feeding began.

Startle response was measured after the flashing of the overhead light for approximately 3 seconds. Flashing lights was the stimulus used in the experiment this one was modeled after (Lucas et al. 2004) and thus, was expected to elicit similar results. The startle response was recorded as one of three categories of behavior: 1) *No response (no observable response in behavior);* 2) *freeze (stop of movement); 3) frenzy (rapid and erratic swimming behavior)*. If the fish continued to swim on the path the observer assumed it was taking or failed to react in any way, the response was recorded as 'none'. If the fish stopped moving completely upon initiation of the startle stimulus, the response was recorded as 'freeze'. If, when the startle stimulus began, the fish began to swim quickly and erratically in a darting fashion, the response was recorded as 'frenzy'. After

the fish were startled, they were left to re-acclimate for ten minutes before post-startle activity level and post-startle mean swim level were recorded. This time period was decided upon after preliminary experiments showed resumption of pre-startle activity levels after approximately 10 minutes.

2.4 Dissections

After each three day trial period, the fish were euthanized using an overexposure to clove oil. The standard fork length was recorded and both liver and brain were removed. The organs were placed in 0.5 mL tubes and immediately submerged in liquid nitrogen. These samples were then transferred to a -80°C freezer for future use.

2.5 Data analysis

Data was analyzed in SAS 9.1 using an ANOVA and a mixed model analysis. Independent variables included fish, strain, day and time of day. Dependent variables included feeding latency, strikes (number of feeding attempts), activity level, mean swim level, post-startle activity and mean swim levels. Interactions were also analyzed. Length data was collected, but was not significant in the analysis. Data was analyzed both by individual experiment and as a complete data set. Because Arlee was present in all experiments, it was hoped that the data set could be presented as one, but due to significant differences in the raw data of Arlee between experiments, this was not possible. Principal components analysis was conducted but no correlations were found to be significant or consistent across all experiments (data not shown). Also, a correlation matrix was made with correlations existing between the two mean swim levels and the two activity levels, pre- and post-startle (data not shown).

Data presented has been normalized to Arlee in order simplify the presentation of the data as well as allowing for all clonal lines to be present on the same graph. The data was normalized to Arlee by subtracting the mean of the three days of each behavioral measure for Arlee from the value for the particular behavioral measure for each of the clonal lines in that respective experiment. Due to unequal sample sizes, Tukey-Kramer adjustment was used.

2.6 Assumptions

The normalization of the data was done with two assumptions. The first assumption is that any changes in behavior across experiments, as evidenced by Arlee, will be experienced by all strains equally. The second is that environmental effects, such as temporal effects associated with the observer, maternal effects or seasonal variations, will remain constant across all experiments.

3. Results

A summary of statistical values can be found in Table 1 which includes F values and p-values for the behavioral measures normalized across all experiments. Results for each behavioral measure below show strain effects and day effects. If day effects are not listed, they were not significant before normalization.

Mean Swim Level (MSL) on day one showed Skamania ($p < 0.0001$) and Swanson ($p = 0.0022$) to be significantly lower in the water column than Arlee (Fig. 1). Day two also had a strain effect: Clearwater ($p = 0.0254$), Skamania ($p = 0.0083$), Whale Rock ($p = 0.0425$) were all lower in the water column relative to Arlee (Fig. 2). On day three, Hot Creek was significantly higher in the water column than Arlee ($p < 0.0001$), while Skamania ($p = 0.0201$) and Whale Rock ($p = 0.0291$) were significantly lower (Fig. 3).

Activity Level showed significant differences by day. Whale Rock was significantly lower than Arlee on day one ($p = 0.0315$), day two ($p = 0.0001$) and day three ($p < 0.0001$). Swanson was also significantly different from Arlee each day, but it was significantly more active ($p < 0.0001$ on all days). Klamath was significantly different from Arlee on days one ($p < 0.0001$) and three ($p = 0.0347$) but all other lines were not significantly more or less active than Arlee ($p > 0.05$). See figures 4, 5 and 6.

Feeding latency had a significant strain effect with Clearwater being significantly different from Arlee ($p = 0.0001$) (Fig. 7), but not a significant day effect (Fig. 8.).

Feeding attempts (strikes) showed a significant strain effect on day three with Clearwater ($p = 0.0012$), Klamath ($p = 0.0104$), Skamania ($p = 0.0243$), Swanson ($p < 0.0001$) all having significantly lower number of feeding attempts than Arlee. See figures 9, 10, and 11.

Startle response showed no significant differences among the lines in regards to type of startle response, data not shown.

Post startle activity level showed a significant strain effect with Swanson being significantly higher ($p < 0.0001$) and Whale Rock being significantly lower ($p = 0.0285$). See fig. 12. There wasn't a significant day effect, Fig. 13.

Post startle mean swim level showed a significant strain effect. OSU was significantly higher ($p = 0.0469$) in the water column and Skamania was significantly lower ($p = 0.0097$) (fig. 14). There wasn't a significant day effect, Fig. 15.

4. Discussion

It was expected that the differences seen in Lucas et al. (2004) would be magnified due to the use of clones in this study in place of the hybrids used in that study.

Some of the same patterns of the Lucas study exist in this study, specifically mean swim level in Arlee, Hot Creek, Swanson and Clearwater. Mean swim level was the one measure that best fit the hypothesis of behavior differences being related to levels of domestication, a significant result reported by Lucas et al. (2004). It was hypothesized that the less-domesticated fish would swim lower in the water column, and in most lines, that was the case. Also, Lucas et al. (2004) found the semi-wild strains, Swanson and Clearwater, to be more similar in mean swim level than the highly domesticated strains, Arlee and Hot Creek. This experiment also found Hot Creek to be similar to Arlee, when compared with Clearwater. Unfortunately, it was not possible to have all four lines used in the Lucas study in one experiment in this study.

Feeding latency is another behavior that fit with our hypothesis. We expected the most domesticated lines to feed with little to no hesitation despite the presence of the hand delivering the food. In Figure 7, only Clearwater is significantly different from Arlee, $p = 0.0146$. While Whale Rock was expected to show the highest feeding latency due to domestication history, it was not significantly different from Arlee, $p = 0.0861$.

The main conclusion of this study is that it is not possible to distinguish domesticated fish from wild fish based purely on domestication history. Or, in other words, duration of domestication (in generations) doesn't cause consistent changes in behavior. One example of a contradiction to the hypothesis is in feeding attempts. Whale Rock is not significantly different from Arlee on any days (day one $p = 0.9986$, day two $p = 1.000$, day three $p = 0.9998$, figures 8, 9, 10) but on day three, all semi-wild lines are significantly different from Arlee (Clearwater $p = 0.0012$, Klamath $p = 0.0104$, Skamania $p = 0.0243$, Swanson $p < 0.0001$). In activity level, Swanson is much higher than the other

strains while Whale Rock is consistently low. Both clonal lines are significantly different from Arlee, but Swanson is more active and Whale Rock is less active. Activity level is one of the measures that changes unpredictably during domestication (Moretz et al. 2007, Robison et al. 2005) and aggression is another (Berejikian et al. 2004, Johnsson et al. 1996, Kelley et al. 2006). Due to its unpredictable nature, it was not one of the measures we had expectations about. It is interesting to see the range of activity levels and how it varies regardless of domestication history.

Looking at feeding intensity, or strikes, Whale Rock is very similar to Arlee on all days. If the domestication process influences this behavior, one would expect it to be consistent across fish with similar domestication history. However, two possible explanations for the varying number of strikes across days are acclimation to the experimental environment and potential differences in learning times.

Comparing the pre-startle and post-startle data shows similar patterns, which suggest that the recovery time of the clonal lines, at least in their behaviors, to be adequate. Another study, looking at stress response and cortisol levels, found recovery time of 1-2 hours for the clonal lines (Drew et al. 2007). It appears that although the behaviors have returned to pre-startle levels, the physiological systems may require more time.

The most surprising result from this experiment was the striking differences in behavior of the Arlee clones across experiments. This can be best explained by looking at the raw data. For example, in experiment one, feeding latency on day one was approximately 13 seconds and in experiment three it was 17 seconds. In experiment two, feeding latency was approximately 23 seconds. Those differences may not seem extreme,

but Whale Rock, which had the highest feeding latency of all the lines, had a feeding latency on day one of 24 seconds. Also, looking at days two and three for experiments one and three, the feeding latency decreased to between five and seven seconds. However, for experiment two the Arlee line did not ever fall below 10 seconds, which is also more similar to the Whale Rock line than the other two experiments with Arlee fish. These differences led to the decision to normalize the data. Lee and Berejikian (2008) showed differences in behaviors among individuals of one species of rockfish with regards to feeding behavior. This is not unexpected as the individuals in that population were genetically distinct. This is just one example of the plasticity that exists in one population of one species. It is interesting to see a similar result in behaviors of individuals with identical genetics. The Arlee clones in this experiment showed the plasticity that exists within one individual. It is not clear what this plasticity can be attributed to but some suggestions might be mitochondrial type (which could also affect development rate), other maternal effects, or differential gene expression.

The normalized graphs present a clear picture of the variation that exists among the clonal lines. It was unfortunate that the lines could not all be tested at one time, but normalizing the data appears to have offered a simple way to look at the overall trends without compromising the data.

There is a great potential for additional future experimentation using the behavioral differences among the clonal lines. For example, some work on gene expression is currently being done on zebra fish, *Danio rerio*, using microarrays (B. Robison, pers comm.,Wright et al. 2006). Tissues were preserved from each fish in this experiment with the hope that further molecular and genetic analyses could be done.

Possible future projects could include microarray analysis to look at expression of specific genes or combining the behavioral data with other measures such as stress response hormones or other important biological elements such as enzymes. Future research could also include QTL analysis for mean swim level and feeding latency. QTLs for certain behaviors have already been found in zebrafish (Wright et al. 2006), as well as other animals including junglefowl, *Gallus gallus*, and White leghorn layers (Jensen and Andersson 2005). A recent study suggests a link between various traits such as stress response and growth rate (Drew et al. 2007). It is likely that behavioral QTLs will be found to be linked to other traits as well. Another possible study would be trying to determine what other traits behavioral traits are linked to, in an effort to maximize the production for commercial hatcheries as well as minimize domestication effects on supplementation programs.

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Chapter 3

1. Summary

This experiment was designed to determine if there were specific behaviors or levels of a behavior that change with domestication time. The results show that it is not possible to predict behavior from domestication history alone. This is not surprising due to the varied background of the fish. Many of the clonal lines originated from very different environments that had unique selection pressures. The geographic distribution of the lines ranges from Alaska to California. This wide range could explain some of the behavioral differences, but it can also explain some of the similarities. For example, Arlee, OSU and Hot Creek are all highly domesticated and looking solely at domestication history, one would expect them to behave similarly. However, they all originate from the same river system as well and it is possible that they are similar because of their origins rather than their domestication history. One example of how environment can lead to specific selection pressures involves the Skamania line, which has been shown to have a higher resistance to the disease agent *Ceratomyxa shasta*, where it is present in the waters in which the fish originated (Ibarra et al. 1994). There are other environmental factors that have been shown to exhibit selection pressures such as levels and types of predation which would have an effect on startle response (magnitude and duration) and recovery time, as well as boldness, water temperatures and flow regimes that would affect development rate and time of emergence, food type and availability which would possibly have an effect on activity level and/or aggression.

In addition to the endemic environment, the fish did also differ slightly in genotype. A previous study on some of the lines has shown there are differences in development rate between the lines (Robison et al. 1999). It was shown that Swanson and Clearwater develop from fertilization to hatch faster than Arlee and OSU. This is likely

one of the factors that is responsible for differences that exist among the lines, at least at early ages such as swim-up. These differences in behaviors, such as number of strikes which would be a measure of feeding intensity and a sign that the fish are more developed internally to digest food, are typically not present at adult stages but appear to be more significant in the early stages of development.

The experiment showed differences occur not just between clonal lines, but within the lines as well. One possible explanation for the differences in behavior within clonal lines is phenotypic plasticity. Each fish from the various clonal lines is genetically identical, which means they each contain the same genes from which the same behaviors can be expressed. However, it was shown that Arlee did not exhibit the same levels of behaviors across all three experiments. Because the genetics are the same (except mitochondrial differences) and the environment was the same as well as the methods, it would lead one to believe there is some plasticity in the expression of genes across individuals. Increased phenotypic variation (as measured by fluctuating asymmetry) has been demonstrated in fully homozygous clones (Young et al. 1995) as well as in partially inbred gynogenetic trout (Leary et al., 1985).

Studies on inbred mouse lines have shown similar results in fluctuating asymmetry (Festing 1979). Inbred mice are highly homozygous and it has been shown that as homozygosity increases so do fluctuations in asymmetry. The behavioral fluctuations in the Arlee line could be examples of a similar phenomenon in behavioral phenotypes to previous observations applied to morphological or developmental phenotypes. The Arlee line is one of the oldest domesticated lines and the fluctuations

from experiment to experiment could be an example of the increase in variation that occurs as time in domestication increases.

It's also possible that a small amount of variation can be explained by the maternal component. The eggs are the source of nutrition for the developing embryo and as such can have differing effects on development rate and condition factor. Also, when the eggs were irradiated, the mitochondria remained intact and unharmed, allowing the egg to remain viable. We know different females were used, but it is not clear if the females were all of the same mitochondrial haplotype. It is possible some of the behaviors were altered slightly based on the mitochondrial type because mitochondria are responsible for energy production inside the cells. It is unlikely that all the females were the same mitochondrial haplotype and it has been shown that mitochondrial haplotype affects development rate and oxygen consumption (Brown et al. 2006). However, if the mitochondrial type or nutritional content of the eggs was responsible for large differences, populations would most likely exhibit drastically different behaviors and we know this is not the case. Most of the lines are fairly homogeneous in their behaviors, regardless of the egg source. This has been shown to be consistent with the data from this experiment and the data from Lucas et al. (2004).

In Lucas et al. 2004, four lines were used: Arlee, OSU, Clearwater and Swanson. They found the less-domesticated strains of Clearwater and Swanson to be more similar in their behavioral patterns than they were to the domesticated lines, and the opposite was true as well with Arlee and OSU more similar to each other than to either Swanson or Clearwater. However, only one behavioral measure was constant in both the Lucas study and this study, mean swim level. Although many more lines were involved in this study,

comparing only Arlee, OSU, Clearwater and Swanson, this study found results consistent with the Lucas study. Statistical analysis showed no correlations among the set of behaviors measured in this study. New studies have shown that other species of fish exhibit differences in behavioral syndromes (Lee and Berejikian 2008, Moretz et al. 2007). It is possible that correlations exist among these behaviors and other behaviors that were not measured such as aggression, boldness, courtship behavior or territorial displays. It is also possible that there are correlations between suites of behaviors or behavioral syndromes that exist in rainbow trout.

If supplementation is to be the only method to revive declining populations of salmonids, it is imperative that the rearing of the fish change such that the fish produced are better fit for the wild environment. Many reviews have been published that address that very issue (Huntington, 2004, Naish et al., 2008, Taylor, 1991). It is apparent that the issue of domestication has become extremely important, which can hopefully lead to the production of fish that are more successful upon release and ultimately, increases in the wild populations.

2. Potential Future Projects

The genome map for trout is not complete yet, although partial maps exist (Gharbi et al., 2007, Guyomard et al., 2007, Nichols et al., 2003, Young et al. 1998) and QTLs linked to cortisol levels have been discovered (Drew et al. 2007). Continuing to map the genome with QTLs for behavior would be beneficial.

Another avenue would be to look at the possibility of maternal effects for behavioral and other traits by using irradiated eggs from different females with different mitochondrial haplotypes and fertilizing them with milt from a single clonal line. Ideally,

that could be done in all the clonal lines, but the initially the most divergent lines would most likely show the biggest differences. Behavioral measures could be taken as well as other measures such as stress response via cortisol levels, aggression levels, development rate and others.

Creating doubled haploids between divergent lines, such as Arlee/Whale Rock or Swanson/Clearwater could potentially provide additional data to support their divergence. It would also be possible to look for QTLs for mean swim level and feeding latency. Those were the measures that appeared to be fairly consistent with domestication history.

Finally, other measures combined with behavioral measures would lead to a more complete picture of the various clonal lines. Other measures could include levels of growth hormone or other hormones, as well as presence or absence of particular enzymes.

	Strain	Day	Strain * Day
Activity Level	56.55,<0.0001	4.04, 0.0187	1.75, 0.0466

Mean Swim Level	20.85, <0.0001	10.60, <0.0001	2.57, 0.0018
Feeding Latency	4.32, 0.0002	2.98, 0.0526	1.47, 0.1228
Strikes	2.80, 0.0095	28.13, <0.0001	3.54, <0.0001
Activity Post-Startle	13.50, <0.0001	2.99, 0.0520	0.94, 0.5186
Mean Swim Level Post-Startle	7.40, <0.0001	2.11, 0.1232	0.76, 0.7150

Table 1. Summary of behavioral data for rainbow trout clonal lines from all experiments combined. Reported are F values followed by p values for each behavioral measure.

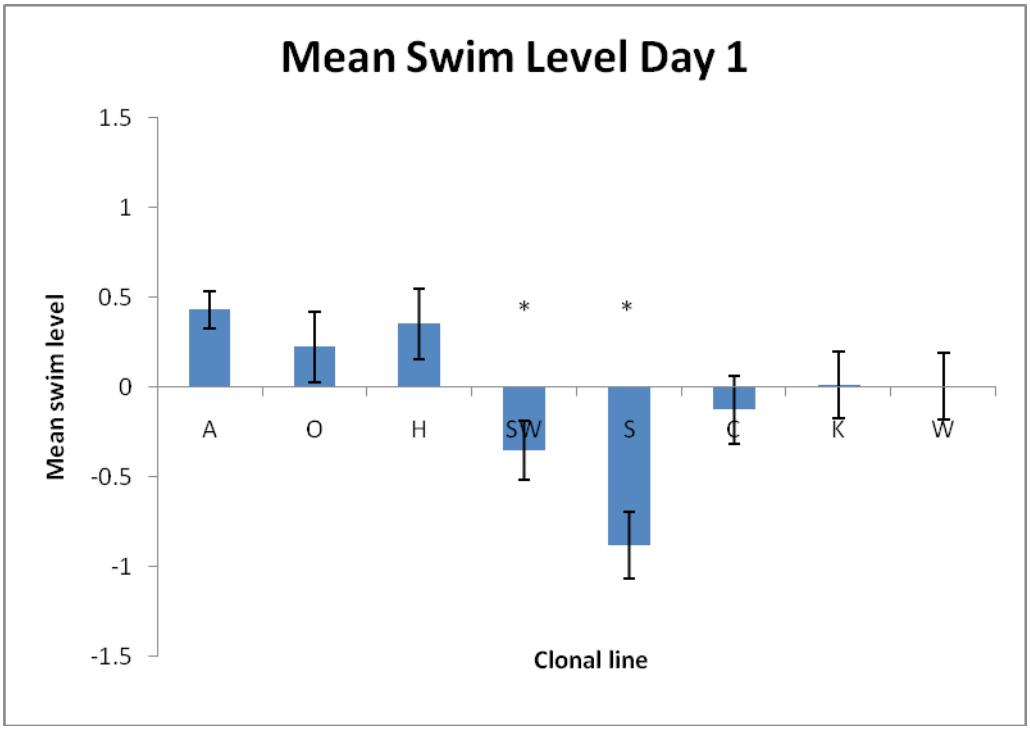

Figure 1. Mean swim level by rainbow trout clonal line for day 1. Clonal lines that were significantly different from Arlee are designated with an asterisk.

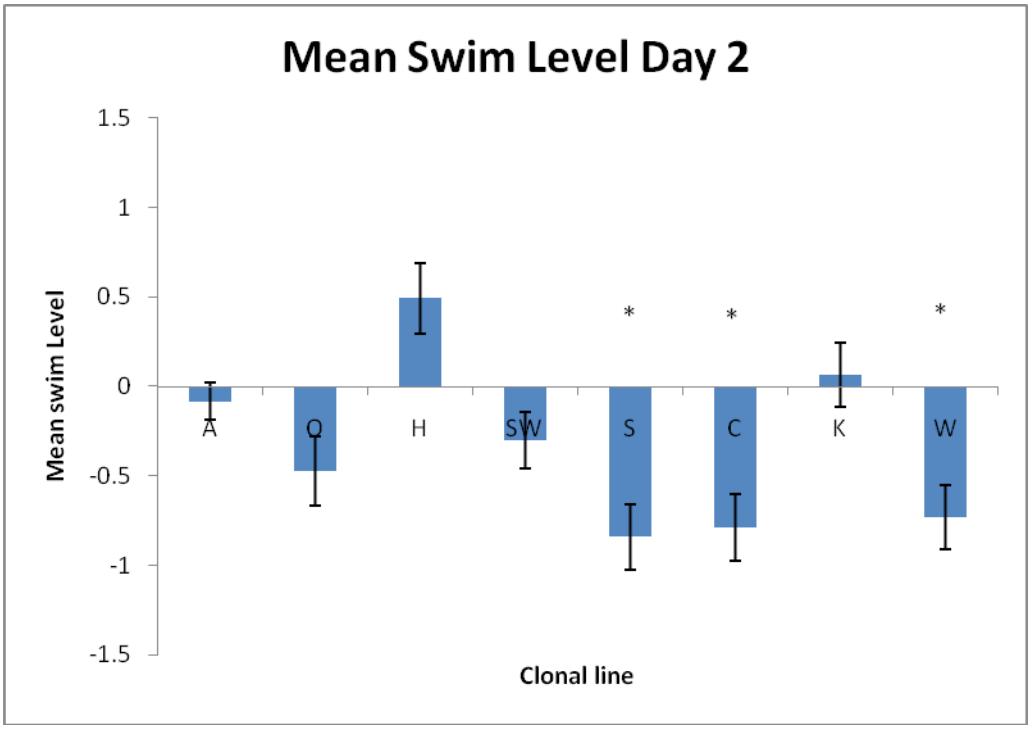

Figure 2. Mean swim level by rainbow trout clonal line for day two. Clonal lines that are significantly different from Arlee are designated with an asterisk.

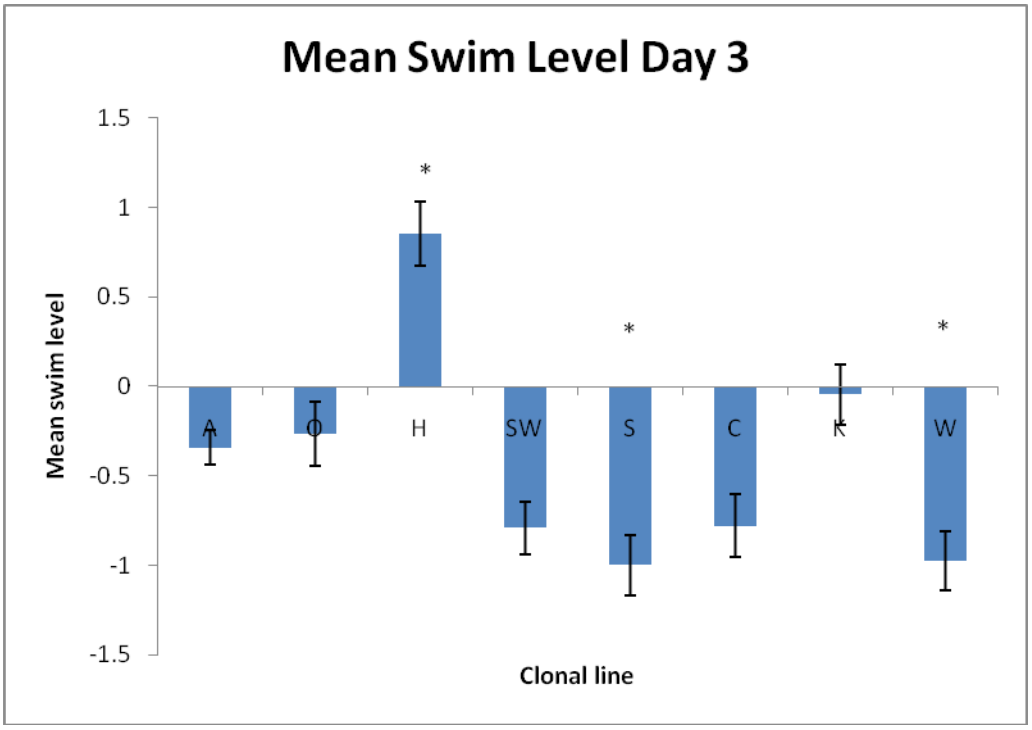

Figure 3. Mean swim level by rainbow trout clonal line for day three. Clonal lines that are significantly different from Arlee are designated with an asterisk.

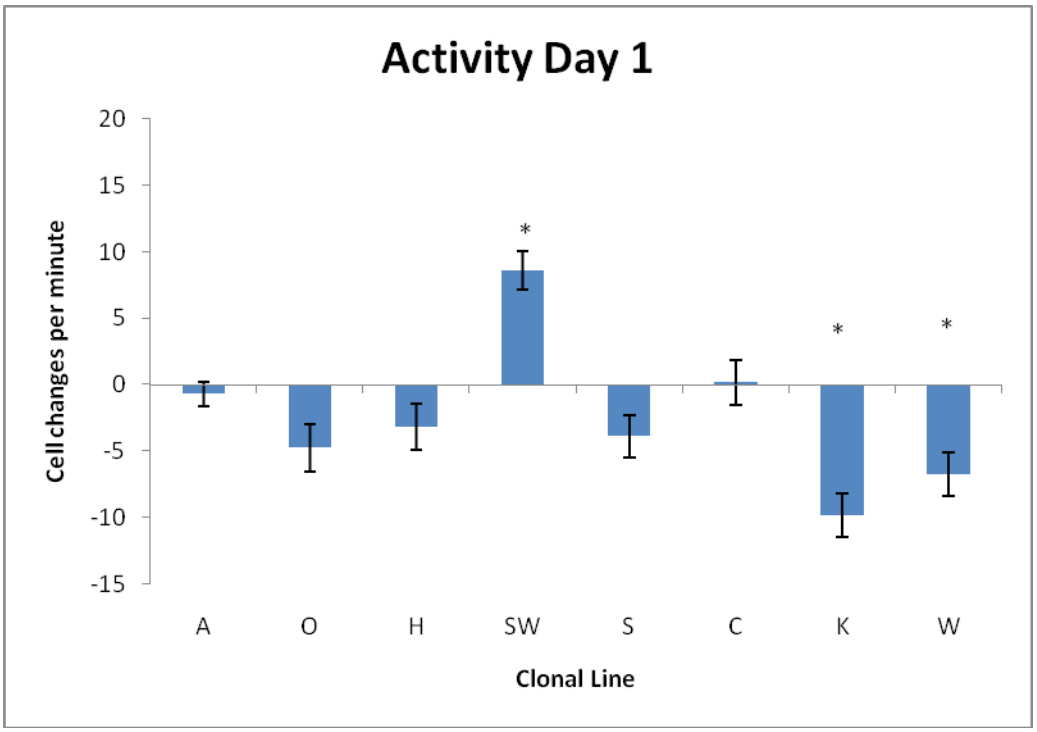

Figure 4. Activity level by rainbow trout clonal line for day one of observations. Clonal lines that were significantly different from Arlee are designated with an asterisk.

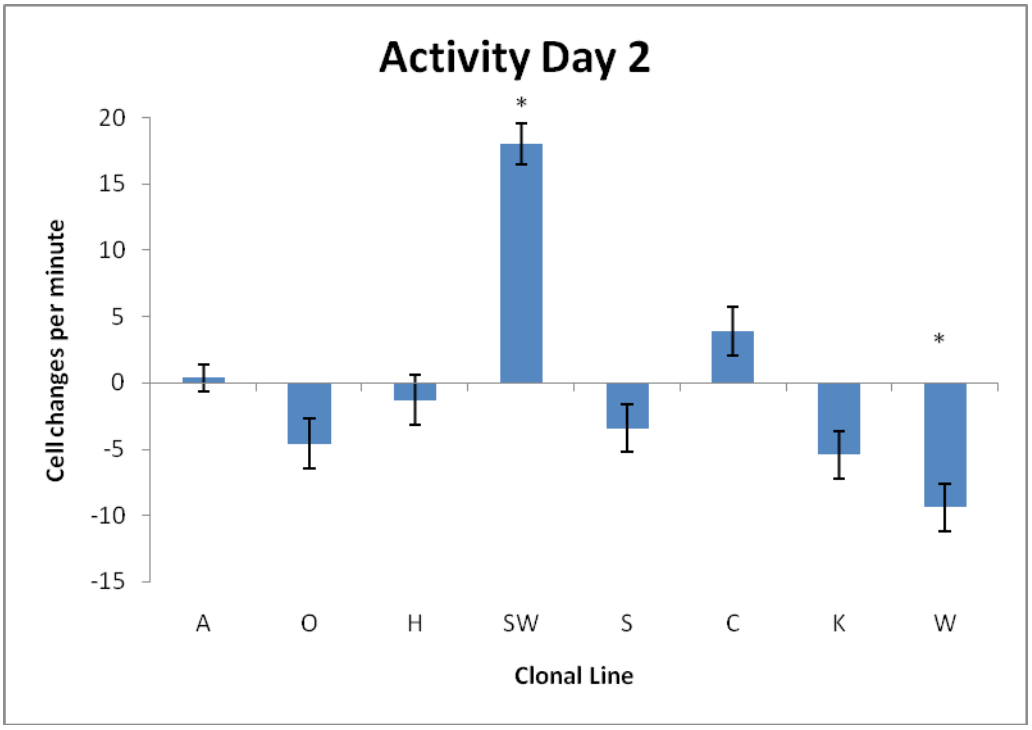

Figure 5. Activity level by rainbow trout clonal line on day two of observations. Clonal lines that were significantly different from Arlee are designated with an asterisk.

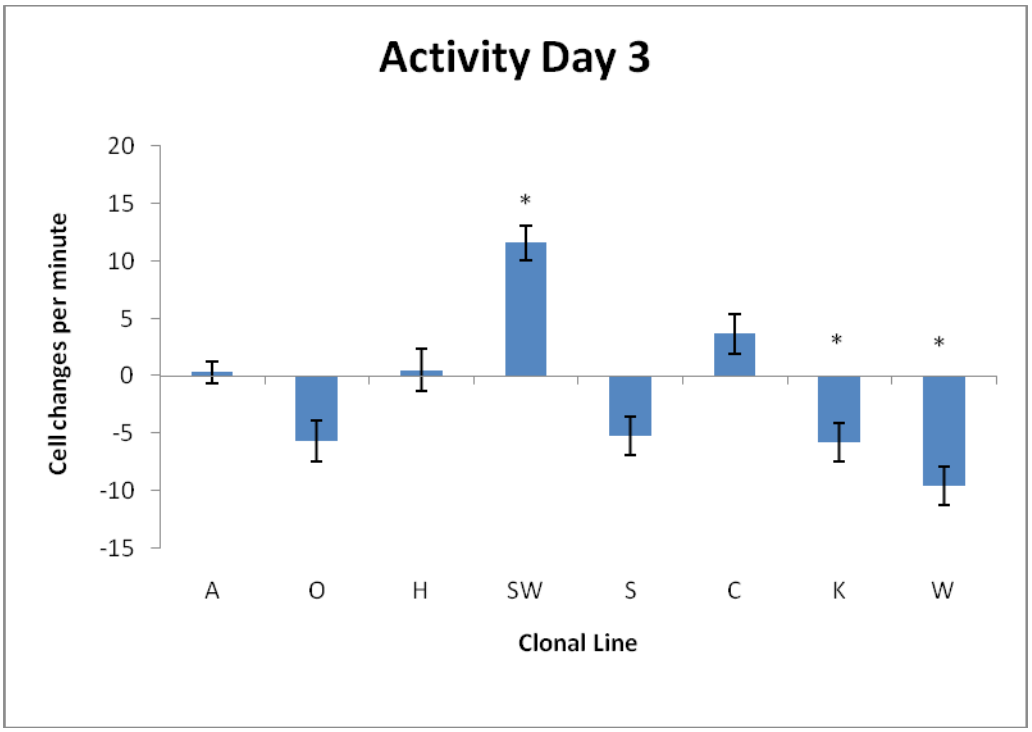

Figure 6. Activity by rainbow trout clonal line on day three of observations. Clonal lines that were significantly different from Arlee are designated with an asterisk.

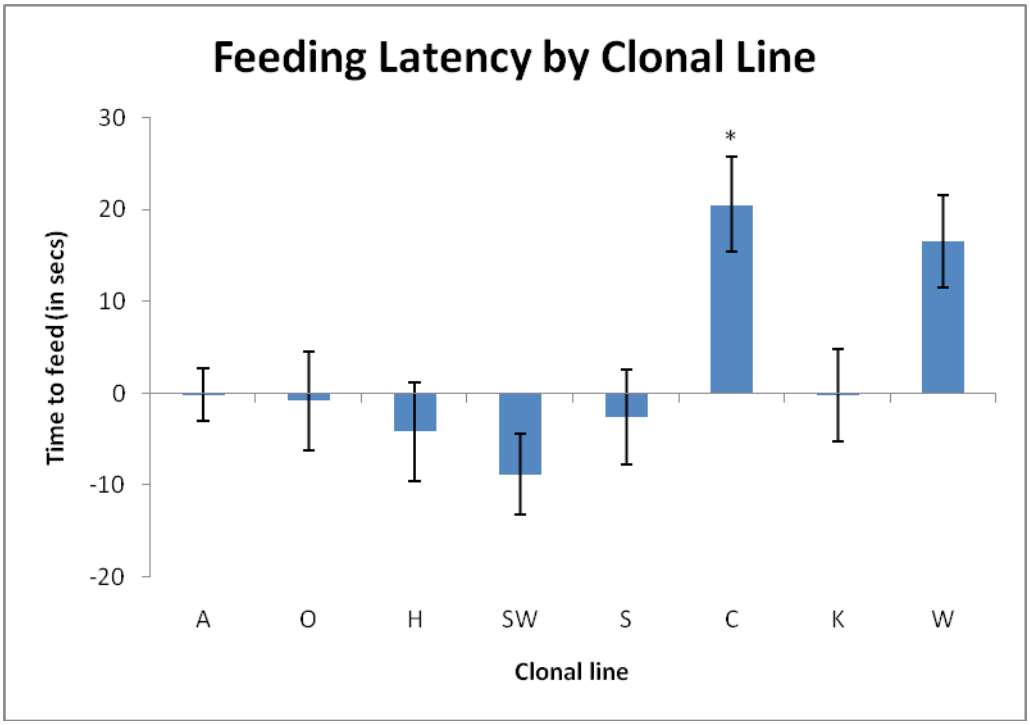

Figure 7. Feeding latency by rainbow trout clonal line. Clonal lines that were significantly different from Arlee are designated with an asterisk.

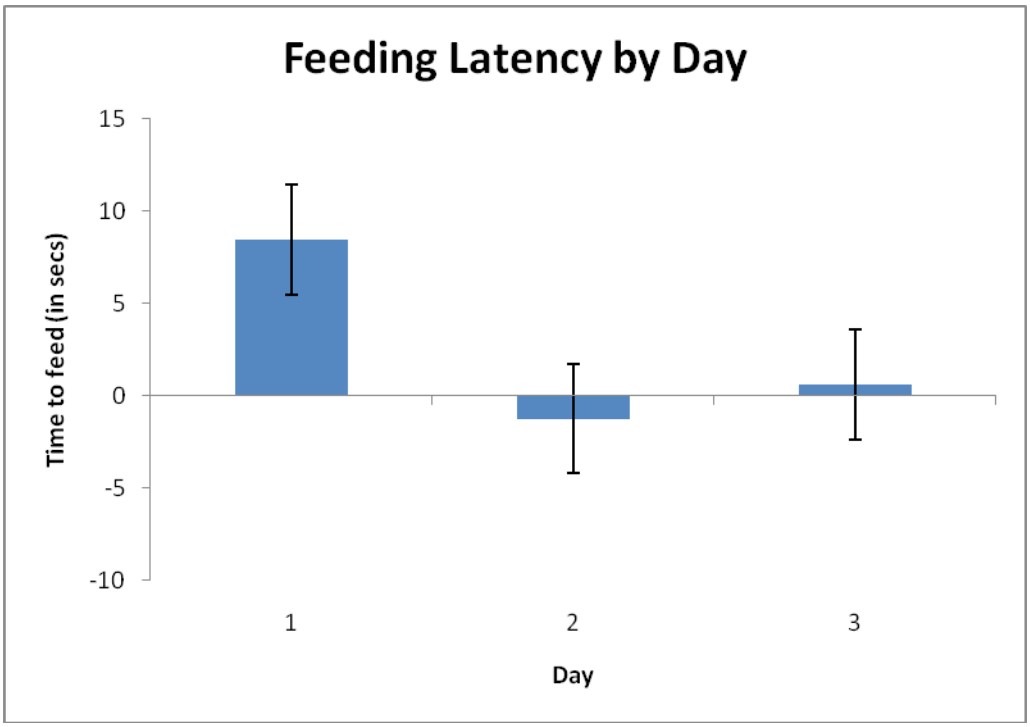

Figure 8. Feeding latency by day. There were no signicant differences between days, although day one and day two are the most different, p = 0.0576.

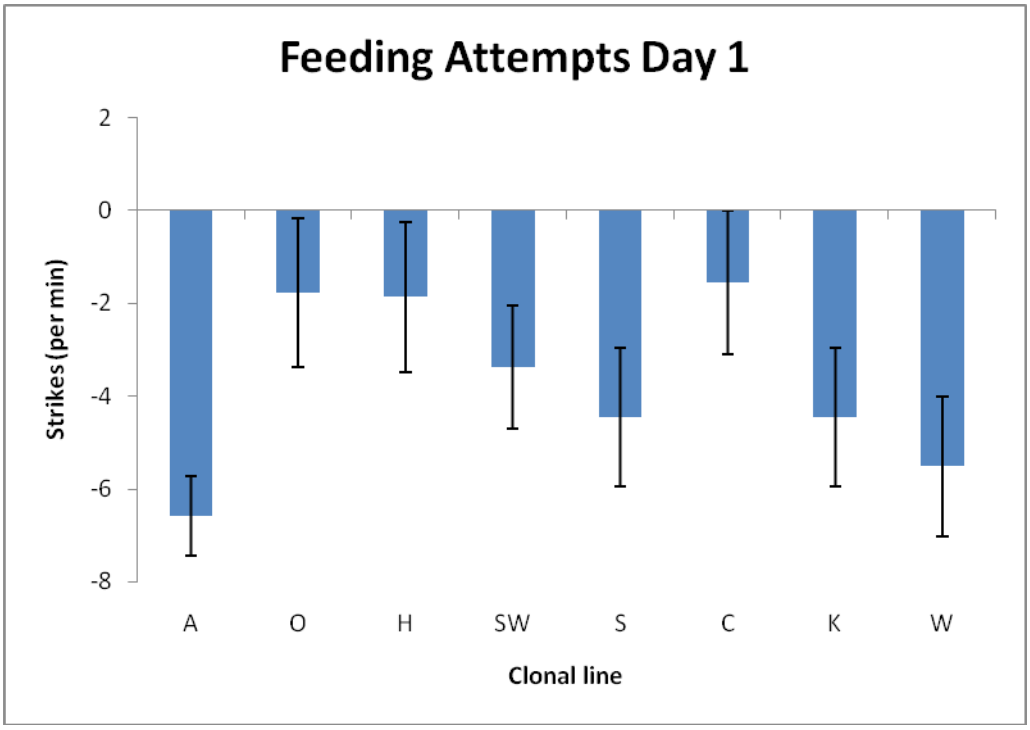

Figure 9. Feeding attempts on day one of observations.

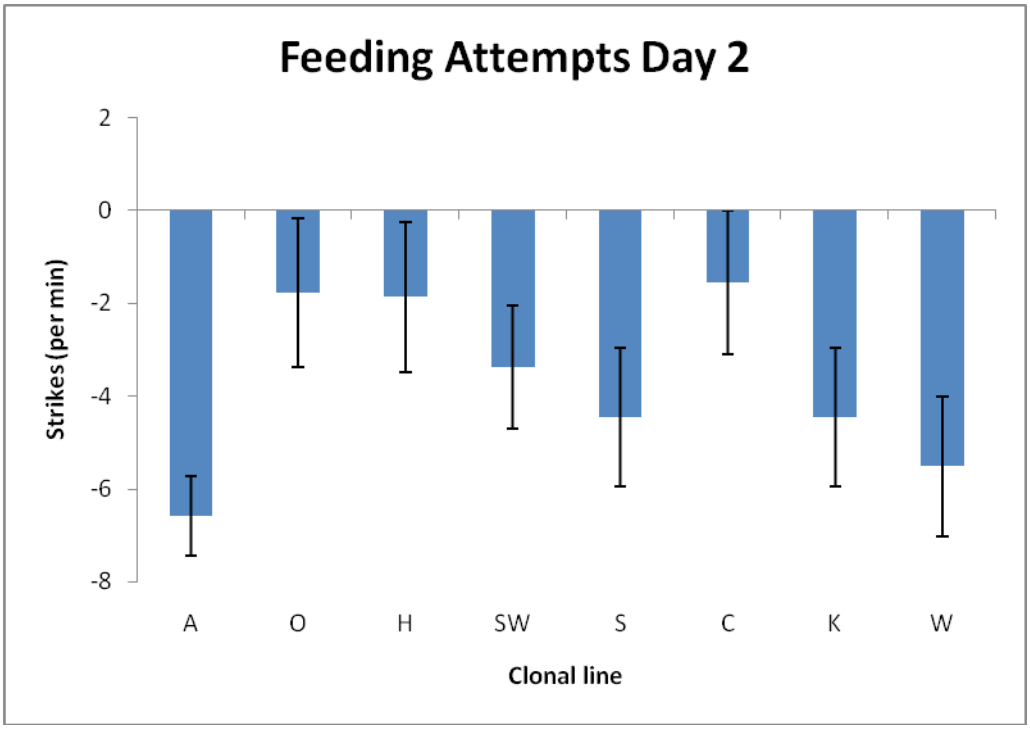

Figure 10. Feeding attempts on day two of observations.

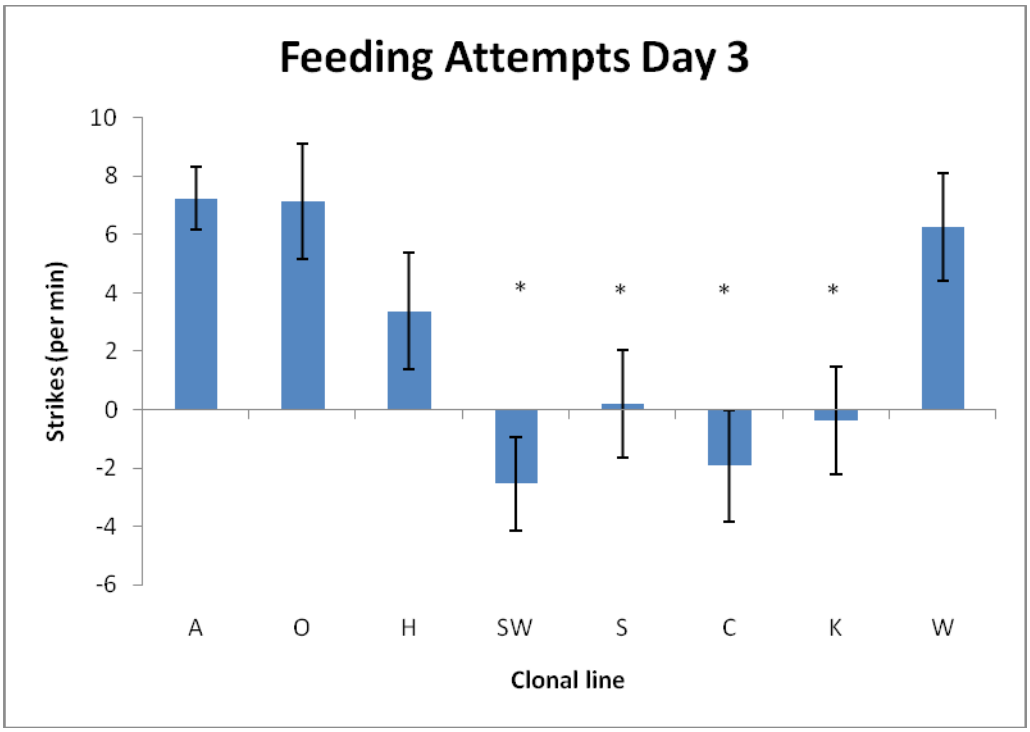

Figure 11. Feeding attempts on day three of observations. Clonal lines that were significantly different from Arlee are designated with an asterisk.

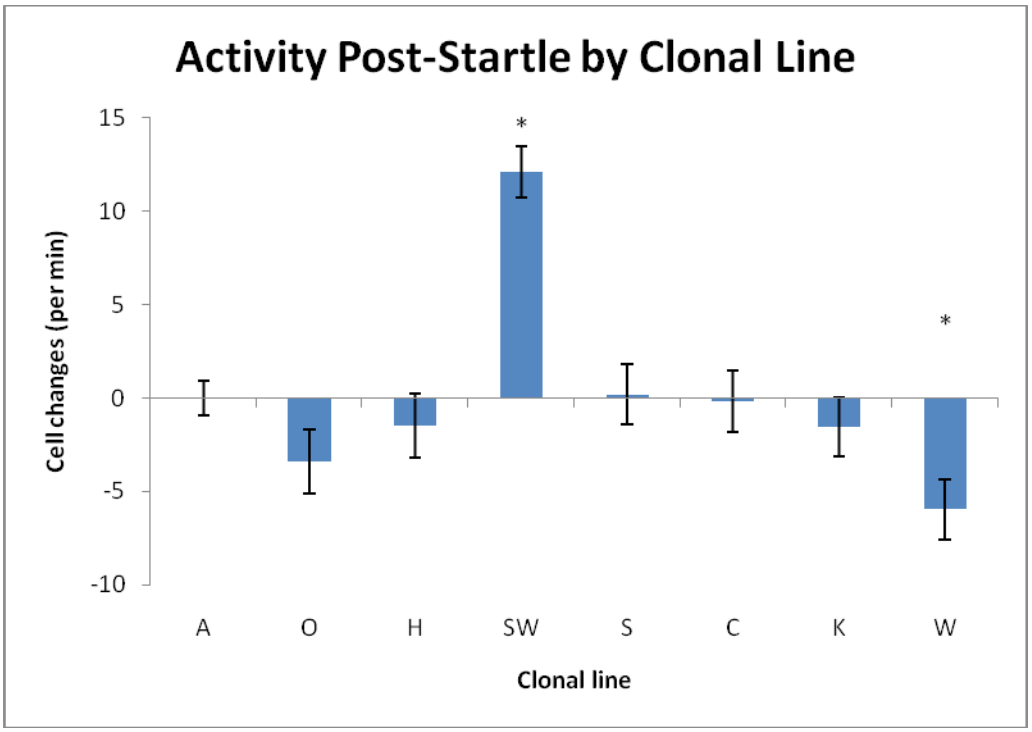

Figure 12. Post-startle activity level by rainbow trout clonal line. Clonal lines that were significantly different from Arlee are designated with an asterisk.

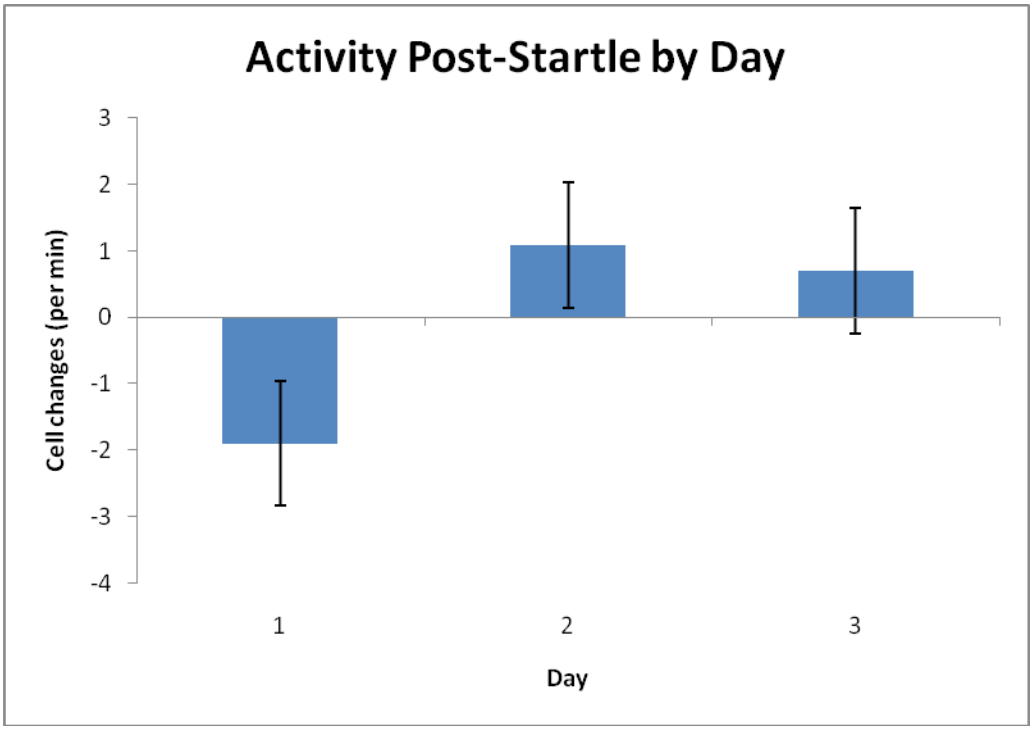

Figure 13. Post startle activity level by day. There were no signicant differences between days.

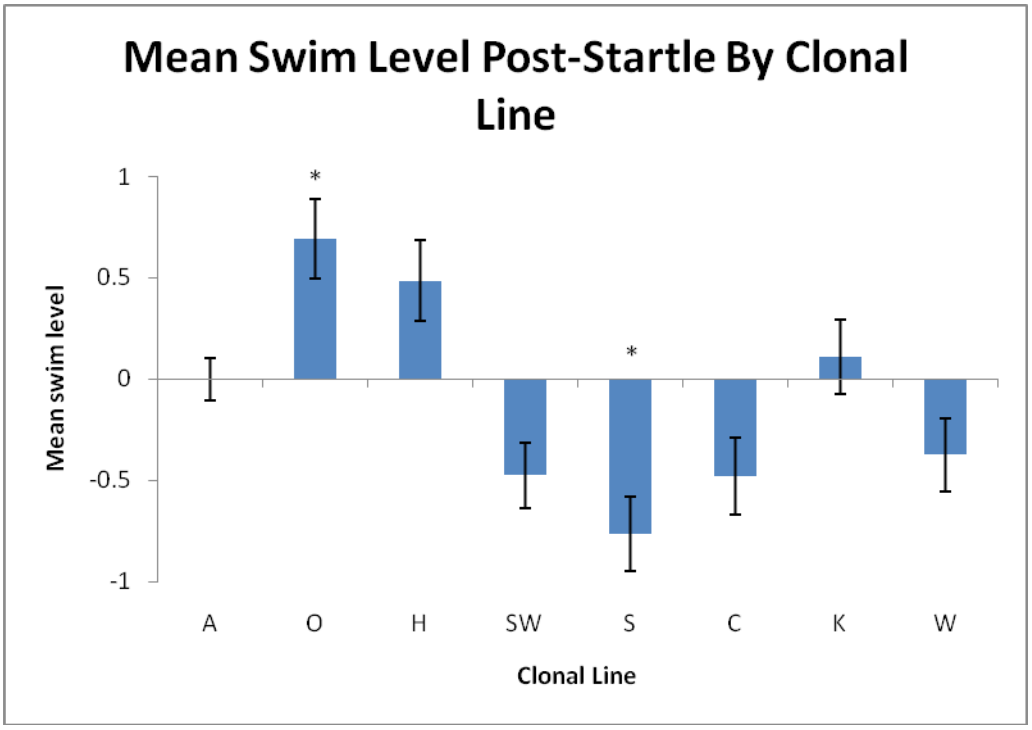

Figure 14. Post-startle MSL by rainbow trout clonal line. Clonal lines that were significantly different from Arlee are designated with an asterisk.

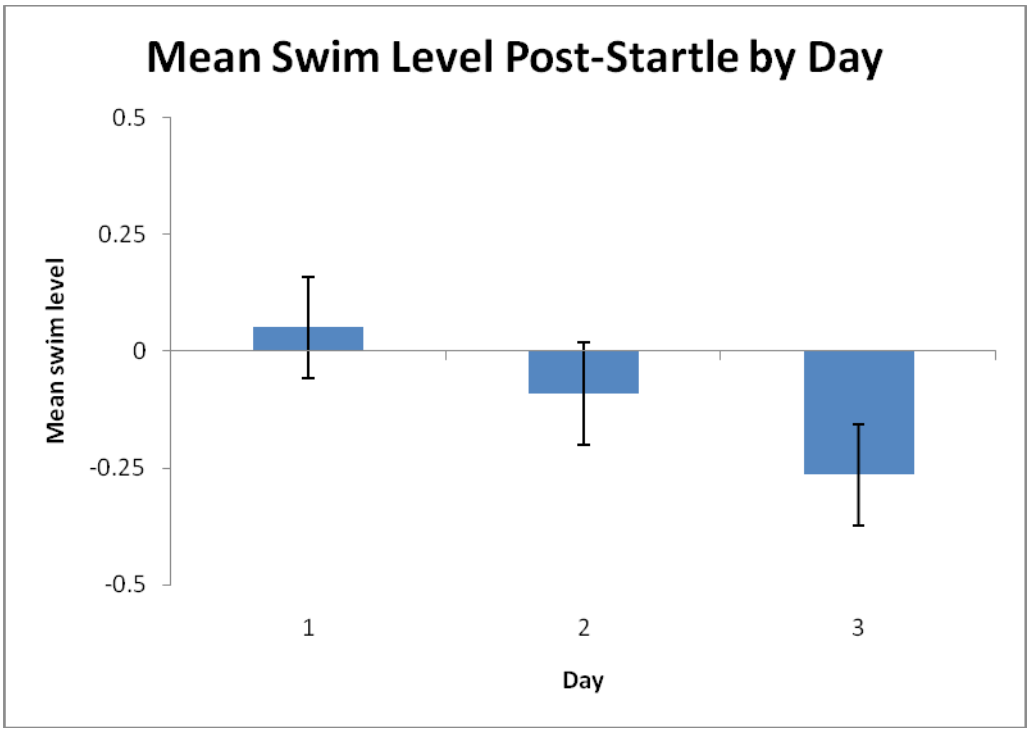

Figure 15. Post-startle MSL by day. There were no significant differences between days.

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