AN ABSTRACT OF THE THESIS OF

Katharine E. Self for the degree of Master of Science in Fisheries Science presented on May 17, 2017.

Title: The Effects of Tank Structure and Egg Size on the Production of Wild Steelhead (Oncorhynchus mykiss) Surrogate Fish.

Abstract Approved:

_____________________________________________________________
David L.G. Noakes

One of the biggest hurdles for a juvenile salmonid (Oncorhynchus mykiss) is migrating downstream from freshwater spawning grounds to the ocean. Juveniles from wild broodstock were reared from the South Santiam River for more than 1 year at the Oregon Hatchery Research Center (OHRC) in Alsea, OR. The fish were reared for 9 months on two treatments: conventional tanks and tanks with a scalable complex structure that was easy for hatchery staff to implement and clean. Both groups were reared at densities below conservation hatchery standards and fed low-lipid experimental diets. Assessment of fish quality included morphometric measurements, behavioral assessments, and growth rate analyses. Fin morphometrics showed the dorsal and caudal fins of fish reared in tanks with structure had less fin degradation than fish reared in conventional hatchery tanks. The two treatments also provided fish for behavioral assessments, including predator-avoidance and foraging behavior patterns. A separate third component tested fish growth rate related to the egg size of individual at spawning. It was shown that fish originating from smaller eggs (within a single female) grow at a faster rate than larger eggs. The goal of this project is to improve on current hatchery practices to produce a wild “surrogate” fish for tagging studies when a wild run is not large enough to provide experimental animals.
The Effects of Tank Structure and Egg Size on the Production of Wild Steelhead (*Oncorhynchus mykiss*) Surrogate Fish

by

Katharine E. Self

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

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

Katharine E. Self, Author
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CONTRIBUTION OF AUTHORS

Dr. Eric Billman assisted with data collection and project design for all chapters.

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CHAPTER 1 - GENERAL INTRODUCTION

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Salmonids in the Willamette Basin

One of the biggest challenges for a juvenile anadromous salmonid is migrating downstream from its freshwater rearing grounds to the ocean (Romer, Leblanc et al., 2013). For example, survival can be as low as 30% through the estuarine environment for salmonid smolts in Oregon coastal rivers (Johnson, Power et al., 2010). Add one or more dams and reservoirs that must be successfully navigated to this equation and survival rates can decrease even further (Hostetter, Evans et al., 2012). The goal of my thesis research was to monitor the behavior, survival and life history characteristics of offspring of naturally-produced winter-run steelhead, *Oncorhynchus mykiss*, reared in an experimental hatchery environment compared to their siblings that were released to emigrate from the Willamette River system.

Specifically, the North Santiam River above Detroit Reservoir in western Oregon, USA is of particular importance because it is one of the few remaining historical habitats for winter-run steelhead within the evolutionarily significant unit (ESU) that at present does not contain winter steelhead (Tinus & Friesen, 2010). By raising juvenile surrogate fish under controlled conditions, we can conduct rigorous testing of alternative rearing conditions that will increase the likelihood of developing adequate structural or operational changes in hatcheries to enhance the future management of winter-run steelhead. Our experimental approach is in response to the Upper Willamette River Conservation and Recovery Plan for Chinook Salmon and Steelhead (ODFW, 2011), which emphasizes the need for successful reintroduction of steelhead above Willamette project dams because
the majority of spawning habitat for anadromous salmonids is upstream of those projects.

For most salmonid populations in the Pacific Northwest of the United States, hatcheries have played a critical role in mitigation and supplementation efforts since they were first established in the 19th century (Behnke, 2010). In the last 25 years, there have been questions as to whether conventional hatchery efforts can produce fish that can enhance populations to or supplement the wild populations that once flourished in the region (Allendorf, Bayles et al., 1997; Nehlsen, Williams et al., 1991; Waples & Hendry, 2008). Consequently the emphasis of hatchery operations has changed from measures of quantities of fish produced to the quality of those fish (typically measured by their performance when they have been stocked in the wild). In response to this, targeted projects have been implemented to produce higher quality fish in a hatchery environment by trying to mimic those conditions experienced by wild fish. The assumption is that fish reared in this manner will have physiological and behavioral characteristics more similar to those observed in the wild (Zydlewski & Johnson, 2002). It should be noted here that my thesis, guided by this assumption, contributes to a greater body of ongoing work being conducted by the Wild Fishes Surrogate Project at Oregon State University where, when possible, natural-origin broodstock are used in hatchery manipulations. (Noakes, Schreck et al., 2013; OHRC, 2016).
Steelhead background

Steelhead trout are the anadromous life history form and rainbow trout are the freshwater resident form of the same biological species (Oncorhynchus mykiss). Both life history forms are iteroparous and capable of repeated spawning events (Ohms, Sloat et al., 2013; Thomas P. Quinn, Vøllestad et al., 2004). A number of populations of steelhead are listed or are of special concern throughout their native range (Good, Waples et al., 2005). In the Pacific Northwest, steelhead populations are classified by their timing of river entry, with two main run types, winter and summer. Summer steelhead adults enter the river during the summer and remain in freshwater until spawning occurs in the winter months. Winter steelhead adults enter rivers during late autumn or winter and spawn soon afterwards. Summer steelhead may spend over 9 months in freshwater before spawning (Robards & Quinn, 2002; Salinger & Anderson, 2006).

Juvenile salmonids undergo dramatic ontogenetic changes in physiology, morphology and behavior when they move downstream from their natal freshwater habitat to the ocean, where they remain for up to 3 years before they return to spawn in their natal freshwater streams to complete their life cycle (Hartman, 1965; Hoar, 1976; Thomas P Quinn, 2011; Yamauchi, Ban et al., 1985).

To observe movement through a river system, tagging studies have been conducted with salmonids reared in conventional hatchery environments including steelhead in the North Santiam River, Oregon (Monzyk, Romer et al.,
2012). Of these, numerous studies have documented significant differences in morphology (Carl & Healey, 1984; Kihsslinger & Nevitt, 2006; Tiffan & Connor, 2011; Wessel, Smoker et al., 2006), physiological responses to stress (!!! INVALID CITATION !!!; Congleton, LaVoie et al., 2000; Jepsen, Davis et al., 2001; Woodward & Strange, 1987), disease susceptibility (Hedrick, Adkison et al., 1998; Mazur & Iwama, 1993; Salonius & Iwama, 1993), predator avoidance (Fritts, Scott et al., 2007), and territorial behavior (Metcalf, Valdimarsson et al., 2003) as well as early development (Hansen & Jonsson, 1991; Shrimpton, Bernier et al., 1994) and survival (Chittenden, Biagi et al., 2010; Fritts, Scott et al., 2007; Magnusson & Hilborn, 2003) between hatchery- and naturally-reared salmonids. Hatchery-origin steelhead differ from wild fish in a number of measures at juvenile life history stages (Hill, Zydlewski et al., 2006; Kostow, 2004; McLean, Bentzen et al., 2004).

Manipulation of the hatchery rearing environments has been shown to enhance development of salmonid characteristics (growth, physiology, seawater tolerance) relative to normal hatchery rearing protocols (!!! INVALID CITATION !!!; B. Ola, Davis et al., 1995; Seals Price, 2003; Zydlewski, Fott et al., 2003). Other rearing practices can alter appearance (fin condition) and performance (fitness) of steelhead (Barry Berejikian, 2005; B. A. Berejikian, Larsen et al., 2012). It is possible that these reported differences could influence migration behavior of hatchery fish, potentially confounding estimates of dam passage efficiency and survival. It will be necessary to understand how the differences
might either affect interpretation of results from dam passage studies using them or, alternatively, how hatchery rearing procedures might be altered to decrease the differences between hatchery-origin and wild-caught fish.

The Wild Surrogate Project is important because it falls at an intersection of fish-human interactions. Although the Detroit Reservoir Dam can produce up to 100,000 kilowatts of energy vital to the surrounding areas, as previously mentioned there is currently no wild-run of winter steelhead present above the dam in native spawning grounds (OHS, 2017) By using the wild surrogate fish reared under experimental conditions, we hope to determine which alternative methods of hatchery rearing as well as dam construction and operation will best increase the survival of wild fish in the future.

**Experimental Hatchery Environment**

Our approach is informed by previous studies such as one at Coleman National Fish Hatchery, CA, USA, which showed that one-year steelhead trout reared in ponds had higher growth hormone levels and tolerated seawater transfer better than fish reared in conventional raceways. Factors such as feeding conditions, structure, and fish density in the pond that differed from raceway conditions were hypothesized to have contributed to the naturalization of the hatchery environment resulting in more physiologically competent smolts (Zydlewski & Johnson, 2002). Likewise, our study differs from conventional aquaculture or hatchery studies not only in our experimental manipulations, but also from standard rearing practices at our facilities. These practices include
lower than conservation hatchery rearing densities, experimental diets meant to mimic diets found in the wild, and study specimens being reared on natural Fall Creek water after incubation (Noakes, Schreck et al., 2013).

A real-world example can be observed by contrasting conventional hatchery practices to those at the Oregon Hatchery Research Center (OHRC). Conventional hatchery conditions generally consist of high fish density rearing environments starting from incubation. Most hatcheries reduce the number of times they must handle fish by moving them from incubators, to troughs where they start feeding by mouth, and then finally to uniform concrete raceways. Fish are stocked into raceways at a fish density of approximately 300 fish m$^{-3}$. In the Pacific Northwest, juvenile anadromous salmonids are typically reared through the smolt stage and then released into stream environments (Zydlewski & Johnson, 2002). The average lipid content (depending on a hatchery’s budget) for conventional feed is around 22 percent (O’Neil, 2017).

Hatchery conditions at the OHRC located on Fall Creek in the Alsea River basin are very different in the density at which they are reared, the type and size of rearing tank, the food which they are fed, and the water they are reared in (D. L. Noakes & C. Corrarino, 2010).

**Density**

Average densities in production hatcheries are higher than conservation hatcheries, which are higher than the densities at which our study fish are held, meaning that the densities implemented at the OHRC are extremely low
Rearing densities can have profound and lasting effects on fish that are eventually released to a natural river system. These differences may include behavior, morphology, growth, and survival of juvenile fish upon release. Banks and LaMotte (Banks & LaMotte, 2002), showed that increased rearing densities produced significant, but inconsistent, reductions in smolt size at release across three brood years.

**Tanks/Facilities**

The rearing conditions at the OHRC were chosen with the well-being of the fish in mind. These concerns include possible effects on fish development due to concrete and rebar constructed raceways interfering with orientation ability. Although there is rebar present in the raceways at the OHRC, and many of the possible disturbing features of conventional hatcheries, we try to mitigate this by rearing study fish away from the rebar raceways in fiberglass tanks (Putman, Meinke et al., 2014). We include a camouflage cover on all outdoor tanks to provide shade, to keep fish from jumping out and to keep predators at bay. Overhead tank covers significantly improve growth and feed conversion rates of trout, and result in significantly enhanced escape responses of trout (Becket & Barnes, 2015; Kientz & Barnes, 2016; Krebs, Barnes et al., 2016; Pickering, Griffiths et al., 1987; Walker, Parker et al., 2016). The circular tank shape allows for a continuous tangential flow that can be easily adjusted to manipulate water velocity with improved self-cleaning performance (Oca & Masalo, 2013).


**Diet**

The custom experimental diets are used in the production of our experimental fish. These Wild Surrogate project diets are designed to more closely mimic natural feeding intake of wild fish than conventional production diets (Barton, Schreck et al., 1988; Feist & Schreck, 1990). Diet components include a lower lipid content (11-12%), fish oil, Spirulina, and lower moisture content (FAO, 2017). These experimental diets began with the need for size selection in hatcheries, which requires more control over growth rates of their fish. In addition, conventional hatchery rearing with aggressive feeding schedules of high lipid diets has led to increases in early maturation in jacks, mini-jacks, and micro-jacks in the Columbia River basin (Larsen, Beckman et al., 2010). Certain additions to fish diets, both commercial and experimental, can increase pigmentation and flesh coloration when raised in outdoor pens (Storebakken, Foss et al., 1987).

Our Wild Surrogate steelhead are started on size 0 commercial feed (Bio-Oregon, Warrenton, Oregon) at the OHRC. For the first weeks of feeding, young fish are fed “presentations” of feed by hand 6-8 times per day to become accustomed to surface feeding. At about 4 weeks post first feeding, the OHRC staff develop feed rates/rations based on the size targets (based on the data collected on wild fish by Oregon Department of Fish and Wildlife) requested by field collaborators, such as the Army Corps of Engineers and the Oregon Department of Fish and Wildlife. The type of feed also changes to experimental
diets which are formulated for us (Rick Barrows and Wendy Sealy, US Fish and Wildlife Service, Bozeman, Montana) specifically to have lower lipid levels than commercial feeds - to produce lower growth rates and lower body lipid content in the fish (USDA, 2017). Although these experimental diets are approximately 10 times the cost of commercial feeds, the Project has shown them to be significant in improving smolt quality of the fish we provide to collaborators.

Feed is weighed out based on the desired %BW/day (usually ~3%) for the entire week for each tank of fish. At the beginning of the week, we feed daily to satiation and continue feeding to satiation each day of the week until there is no feed remaining (typically 3-5 days of feeding followed by 2-4 days of fasting). This feeding practice reduces the size bimodality commonly found with aquaculture rearing of salmonids (J. Thorpe, 1977), and more efficient growth rates among the fish (unpublished observations).

Growth Trajectory

It is important to mention here that, according to the Wild Surrogate Project’s winter-run steelhead rearing protocol, comparable data available for growth and size at age of wild steelhead growth are minimal. As such, we are limited in our ability to mimic complete natural growth trajectories. To achieve the target fish size at the specified dates, we have relied on the ODFW hatchery growth program developed by Jean Paul Lagasse over 20 years ago (J.P. Lagasse, 1990; O'Neil, 2017). However, this creates a linear growth rate with little seasonal change. Fish in the wild are likely to experience natural seasonal
variation in growth rate (Bacon, Gurney et al., 2005; Yard, Korman et al., 2015). The Wild Surrogate Project continues to update its protocols as results of current experiments and more field data from wild steelhead become available.

_Fall/Carnes Creek water_

Fertilized eggs, once transported from the spawning location to the OHRC, are raised to emergence in chilled Heath trays on single-pass, filtered Carnes Creek water (temperature varies seasonally, mean 47°F, range 42°-52°F). They are then transferred to baskets placed in flow-through troughs in the wet lab at the OHRC, provided with single-pass water from Fall Creek (seasonal variation in temperature). At the appropriate time after first-feeding, the fish are ponded into circular tanks outdoors (described above). These outdoor tanks receive single-pass Fall Creek water, which allows for the fish to experience a natural temperature regime as well as limited sediment fluctuations (most silt and sediment is filtered out by a settling pond and rotating drum filter before reaching the tanks).

**Study Setup**

All research took place at the Oregon Hatchery Research Center, located on Fall Creek, a tributary of the Alsea River, in the Coast Range of Oregon. The Mission of the OHRC is to: 1. Understand mechanisms that may create differences between hatchery and wild fish, 2. Develop approaches to manage
the differences to meet fishery and conservation goals, 3. Help Oregonians understand the relationships among wild fish, hatchery fish and the surrounding environment (D. L. Noakes & C. Corrarino, 2010). Note, the OHRC is one of two facilities responsible for our Wild Fish Surrogate research. In addition to the OHRC, the Fish Genetics and Performance Laboratory (Smith Farm) in Corvallis, OR is used for research consistently and is supervised by the Oregon Cooperative Fish and Wildlife Research Unit Leader, Dr. Carl Schreck. All research for my thesis was carried out with the approval of the Institutional Animal Care and Use Committee of Oregon State University (ACUP # 4289).

By asking questions that reflect the OHRC and the Wild Steelhead Surrogate Study’s goals, I intended to improve the quality of deliverable fish to the US Army Corps of Engineers (USACOE) to use in field research projects (Noakes, Schreck et al., 2013). Specifically, the Wild Surrogate Project focuses on improving downstream passage of juveniles through USACOE projects in the Upper Willamette River. This is a high priority because the majority of spawning habitat for anadromous salmonids is upstream of the Army Corp’s projects. Therefore, juveniles must be able to successfully navigate reservoirs and pass through dams.

Goals and Objectives
To investigate whether it is possible to produce a fish with wild-like characteristics in a modified hatchery environment, my research was carried out as follows:

**Chapter 1:** Determine if egg size at time of spawning influences the subsequent growth rate of surrogate fish.

**Chapter 2:** Determine if artificial in-tank structure improves surrogate fish quality over a 6-month period (as measured by body shape and fin condition).

**Chapter 3:** Determine if artificial in-tank structure influences foraging and predator-avoidance behavior of surrogate near date of release.

In Chapter 1, I aimed to determine if egg size within an individual female *O. mykiss* significantly varied in size, followed by a study to monitor the growth rate of the offspring each female’s smallest and largest embryos. To do this I took dimensions of individual eggs from each female to separate them into large and small size groups. Upon emergence, the offspring were tagged using visible implant elastomer and their growth was recorded over the next 8 months. In Chapter 2, a separate study, I reared Wild Surrogate juvenile fish in tanks with or without a complex structure present. Again, using morphometric landmarks, I analyzed the growth and fin quality of the two groups. Finally in Chapter 3 I exposed a subsample of the two groups to behavioral trials to assess the fish’s foraging ability of live prey and their use of cover in the presence of a simulated predator.

**Literature Cited**


CHAPTER 2 - EGG SIZE AND GROWTH IN WILD BROODSTOCK STEELHEAD (ONCORHYNCUS MYKISS)

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Introduction

Many fish species have variation in egg size not only between females, but also within a single female (T. D. Beacham & Murray, 1987; T. D. Beacham & C. B. Murray, 1993; Einum, 2003; C. A. L. Leblanc, Benhaïm et al., 2011). Fish from smaller eggs might grow more slowly after emergence, but once they begin exogenous feeding their growth actually surpasses that of fish from large eggs. In this study I investigated the effect of within-female egg size variation as part of the Wild Chinook Salmon and Steelhead Surrogate Project (Billman, Whitman et al., 2014).

The Wild Chinook Salmon and Steelhead Surrogate Project (hereafter, the Surrogate Project) is a collaboration between Oregon State University (OSU), the Oregon Department of Fish and Wildlife (ODFW), and the United States Army Corps of Engineers (USACOE). One of the Project goals is to develop rearing tactics to produce juvenile surrogate fish that closely emulate wild juveniles for use in dam passage studies. The Project provides juvenile Chinook salmon and steelhead trout of hatchery origin to act as surrogates for wild fish where the capture and tagging of wild fish is not possible. These field studies by the USACOE, ODFW and others are on native salmonid populations on the Pacific coast of North America threatened or at risk of extinction (Monzyk, Romer et al., 2012). For example, the natural-origin broodstock used for this study are listed as threatened under the United States Endangered Species Act (ODFW 2016). As a consequence, natural origin juveniles are not available for the required field
studies. Conventional hatchery origin fish are unlikely to be suitable for the field studies, since they differ significantly from wild fish in size, shape, physiology and behavior (Marchetti & Nevitt, 2003; McDonald, Milligan et al., 1998).

The specific project goals under the Surrogate Project include: A) Produc e fish for field collaborators, B) Aim for a “wild phenotype” target (including morphology, behavior, physiology, reproduction) during the production of surrogate fishes, and C) Investigate methods in hatchery circumstances that result in a fish that closely resembles the aforementioned wild phenotype. To investigate these methods in hatchery circumstances, various measures such as tank environment, water temperature, diet, and rearing density are manipulated.

The study area for *O. mykiss* in my portion of the Surrogate Project is the North Santiam River. The North Santiam River is a 92-mile (148 km) tributary of the Santiam River in western Oregon, USA. It drains 766 square miles (1,980 km²) of the Cascade Range on the eastern side of the Willamette Valley east of the state’s capital city of Salem (Figure 1). There are two dams, Big Cliff and Detroit at river mile 46 and 49 (74 and 79 km), respectively, which produce a combined output of almost 120,000 kW of hydroelectricity. Just downstream of these dams is Minto Fish Collection Facility, owned and operated by the USACOE. This state-of-the-art facility was opened in 2013 in response to the biological opinion of the Endangered Species Act which called for a new “safe and effective” fish collection system at Minto (Sharpe, Cannon et al., 2015). Blocked by Big Cliff Dam near Mill City, OR, the lower North Santiam River supports spring Chinook salmon and summer steelhead. In the reaches
upstream of the dam, the river is managed mainly as a stocked rainbow trout (*O. mykiss*) fishery. Near the stream’s source at Santiam Lake in the Mount Jefferson Wilderness, native cutthroat trout (*O. clarkii*), rainbow trout, and brook trout (*Salvelinus fontinalis*) are also found (Darm & Jackson).

Previous knowledge of the stock in question and the goals of the Surrogate Project, led us to ask two related scientific questions: (a) do wild broodstock steelhead in the Surrogate Project produce eggs of different sizes within one female and, (b) do offspring from eggs of different sizes grow at variable rates during early ontogeny (from initiation of exogenous feeding for the next 8 months)?

Informing this hypothesis are numerous studies across fish species about effects of egg size. LeBlanc et al. (2011) suggested that the correlation between Arctic charr (*Salvelinus alpinus*) egg size and juvenile size (larger eggs produce larger juveniles at the time of hatching) persists for up to 1 year after first feeding (Wallace & Aasjord, 1984). In addition, embryos from smaller eggs develop faster than those from larger eggs, suggesting that different timing of development is connected to egg size (Valdimarsson, Skúlason et al., 2002). Their study demonstrated that differences in behavior between small and large Arctic charr juveniles were triggered by egg size, social environment, time, and the interaction of those factors. Since egg size effects were not cancelled out by the effect of social environment but rather interact with the social environment to affect early behavior, I proposed the hypothesis that egg size affects behavior early in development and mobility and foraging of fish later in life.
In addition, in the brown trout (*Salmo trutta*) literature, it is widely accepted that larger females (fork length or spawned weight) at spawning produce larger eggs on average. In *S. trutta* of the same age reared under common hatchery conditions but having a wide egg size range, most of the between brood variance in mean length both 52 and 90 days after hatching can be attributed to differences in egg size (Ojanguren, Reyes-Gavilan et al., 1996). In addition, size of young fish at hatching accounts for a significant amount of the within brood variability in swimming stamina in the clutches with a wide range in fish sizes.

Environmental predictability has often been suggested as a potential cause or catalyst for egg size variation in salmonids (Terry D. Beacham & Murray, 1985; T. D. Beacham & Murray, 1987; T. D. Beacham & C. B. Murray, 1993). Under the imperfect information hypothesis (Koops, Hutchings et al., 2003), the prediction is that unequal allocation of resources within a clutch decreases with increasing mean egg size. They proposed that the unequal allocation of egg size in brook trout (*Salvelinus fontinalis*) is a tactic that females can use to offset the cost of imperfect information in response to the level of environmental predictability (E.L. Charnov, 1993; Eric L Charnov & Charnov, 1982; Lachance & Magnan, 1990). According to them, in female brook trout there is no evidence that egg size variability results from the physiological constraint of the size of the gonads in which eggs develop. However, environmental unpredictability was associated with increases in both within and among female egg size variability (but it did not influence mean egg size).
Additionally, a model proposed by (Thomas P. Quinn, Vøllestad et al., 2004) for the timing of events determining the egg size-egg number tradeoff in salmonid fishes, focused on early life stages. Their model suggests that the number of eggs recruited is determined while the juvenile or smolt stage salmonid is residing in freshwater or early in the marine phase, depending on the species. They also noted that if adverse environmental conditions are encountered, the number of eggs is reduced by atresia.

LeBlanc (C. A. L. Leblanc, Benhaîm et al., 2011) showed that at emergence, juvenile *O. mykiss* from larger eggs were larger as classically reported in salmonids, however this positive relationship between egg size and growth observed at emergence turned into a negative relationship from the fall to the subsequent spring. Thus, egg size affected growth of juvenile steelhead trout beyond a few weeks after emergence and in a way never reported before, i.e. fish coming from smaller eggs became larger than fish coming from larger eggs in both hatchery and wild fish. The higher growth rate of fish from smaller eggs is suggested to result from greater activity if IGF1 in fish from smaller eggs (Bower, Li et al., 2008).

Based on these studies, the objective of my study was to determine if egg size at the time of spawning is associated with the growth rate of Wild Surrogate Project fish. This is done by determining whether the differences in growth rates of surrogate steelhead smolts from the North Santiam river during the first year of life are associated with differences in egg size at spawning.
To investigate this phenomenon in our Wild Surrogate Project broodstock, I tested whether: 1. The growth rate of juvenile steelhead trout differs in offspring from eggs of different sizes and, 2. The growth rate of juvenile steelhead trout is differs between rearing groups (i.e. small-egg origin fish reared with small-egg origin fish, large-egg origin fish reared with large-egg origin fish, or an equal mix of the two groups). The predictions were that small-egg origin offspring would grow more quickly than large-egg origin offspring over time, and that the growth rates of small and large-egg origin fish would differ between fish reared with other fish from small eggs, large eggs, and mixed size egg rearing groups, respectively.

Because differences in the dynamics of embryonic mortality cannot be detected when survival is assessed at a single developmental stage (Wargelius, Furmanek et al., 2015), I determined that this study should be conducted on a protracted scale barring other factors becoming problematic (i.e. density, tank size, abnormal size distribution). Additionally, there can be a delay between the occurrence of a developmental problem and the time when it can be detected (Kleppe, Karlsen et al., 2013). For this reason, my study was carried out over the maximum number of days possible before a non-normal size distribution emerged in the study (213 days).
Methods

Broodstock Acquisition and Incubation

Natural-origin South Santiam Summer steelhead broodstock were spawned in late April of 2014 (Figure 2). The collection event was a collaboration between ODFW, USACE, and OSU. Collection took place at the Minto Fish Collection Facility, which is located downstream of Minto Dam on the North Santiam River. Minto Dam creates an impassable barrier that encourages migrating fish into the facility’s fish ladder (ODFW, 2017).

A matrix spawning was conducted between 7 male and 7 female fish. Following anesthetization, the males were live spawned while the females were kill spawned. Fork length (cm), genetic fin clips (used to isolate genetic material from individual fish) and scale samples were taken from all fish. In addition, otoliths, kidney samples, and ovarian fluid were collected from the female fish only, while a milt sample was collected from the males. After gametes were collected and placed on ice, they were mixed in a mesh container in a chilled bucket of water (in vitro). The matrix broke down to 2x2, 2x2, 3x3 individuals. All fertilized groups were kept separate and transported to the Oregon Hatchery Research Center (OHRC) where they were held in separate Heath trays. The egg groups were isolated from each other until results of the IHNV tests returned negative from the Oregon Department of Fish and Wildlife on May 6, 2014.
Rearing of Brood Year 2014

Once at the OHRC, each matrix cross was kept in a separate mesh basket to incubate in Heath trays. This prevented individual female’s eggs from mixing. All embryos were incubated in temperatures that mimic the North Santiam River’s cool thermal regime (approximately 6.5-7°C). Shocking and picking of dead embryos occurred in late May 2014 at which time the developing embryos for this study were sorted.

Egg Size Determination Protocol

On August 3rd, 2014, after physical shocking (to identify dead embryos) and the removal of mortalities, we examined a representative sample of each female’s embryos by using a photograph of a subset imported into tpsDig software (Rohlf, 2010). This software allowed us to digitize and compute the average egg diameter for each female and egg size variation within each female (Figure 3, Figure 4). The longest diameter of the eggs and the line perpendicular to that was used to calculate each diameter. This analysis showed measurable differences in diameters between large and small eggs within each female.

Then, 50 embryos from each female were individually weighed in a small evaporation plate on an analytical microbalance after being gently dabbed on a paper towel to remove excess water. This measurement was used to quantify the unique variation within each individual female. One of the fish spawned had significantly larger eggs than the other fish, and one fish spawned had significantly smaller eggs than the remaining five fish resulting in these two
individual’s eggs being eliminated from the study (Figure 5). These individuals were eliminated due to the fact that the largest and smallest eggs from individual females were destined to be pooled for the duration of the study into one “large-egg” group and one “small egg” group in order to evenly represent the females included in the study.

_Egg Sorting and Ponding of Juveniles_

Using this individual size information, I sorted 75 embryos from each female’s largest quartile and 75 eggs from each female’s smallest quartile, excluding the median size class in each case. This was to ensure that large and small batches that were pooled after hatching were equally representative of the five females. Each female’s large and small embryos were kept separate in separate mesh isolets in Heath trays until emergence.

At emergence, the two size groups were pooled across all five females and held in two separate covered mesh baskets (one for large-egg origin fish and one for small-egg origin fish) in single pass flow-through troughs in the indoor wet lab of the OHRC. With 75 embryos of each size class per female, there were a total of 375 embryos to represent each group (750 embryos total) which provided a buffer of 150 fish for tagging or other mortalities before ponding outdoors took place.

Following the Wild Surrogate Project protocol, after complete yolk absorption the fish were fed a #0 size feed starter diet (Bio-Oregon) by hand to
each trough. For the first ~4 weeks of feeding, fish were “presented” feed 6-8 times per day to get them accustomed to surface feeding.

**VIE Marking**

On August 25, 2014 we individually anesthetized fish using MS-222 (50 mg/L MS-222 buffered with 125 mg/L NaHCO3 to pH= 7.0) before placing them on a sterile surface. We inserted a visible implant elastomer tag below the skin just behind the anal fin by injecting elastomer as the needle was withdrawn (C. A. Leblanc & Noakes, 2012). We tagged 359 large-egg origin fish (48.2 cm average FL) with orange elastomer and 350 small-egg origin fish (47.3 cm average FL) with green elastomer (NMT, 2017). The mean length of the mark was 2-3 mm. At this point, fish were left to recover in specific groups according to tag type in mesh baskets in troughs indoors at the OHRC until they were ready to be ponded in outdoor 1 meter diameter fiberglass tanks, following standard ODFW fish production procedures.

Within 1 hour of tagging, I recorded three large-egg origin fish and eight small-egg origin mortalities. The evening after tagging, 109 large-egg origin fish perished when they exited the mesh basket they were being held in through and offset lid. I tagged 87 new fish 2 days later to replace them. Overnight on August 25th, 109 large-egg origin fish perished when they exited the mesh basket through an offset lid. On the morning of August 28th, 87 new large-egg origin fish were tagged as replacements.
Outdoor Ponding and Growth

On the afternoon of August 28, 2014 we hand-counted fish to ensure their numbers into 20 L buckets with aerated water before adding them back to explicitly labeled flow-through baskets in the wet lab. The baskets were populated as follows: 2 baskets with 100 large-egg origin marked fish in each, 2 baskets with 100 small-egg origin marked fish in each, 2 baskets with 50 large-egg origin marked fish and 50 small-egg origin marked fish. We returned any extra tagged fish to the stock basket of unmarked fish of their respective sizes.

The newly designated groups were moved outdoors into six 1 meter diameter tanks that were randomly assigned a pre-determined treatment to avoid tank effect. Fish were ponded (moved from indoor rearing troughs to their final 1 meter outdoor treatment tanks for the duration of the study) at the below conservation hatchery standard density of 100 fish per tank. Two tanks contained small-egg origin fish, two tanks contained large-egg origin fish, and two tanks contained 50 small-egg origin and 50 small-egg origin fish. Fish experienced less than 1% mortality/month following ponding.

Fish were hand fed the Wild Surrogate Project low-lipid (8-9%) wild steelhead grower diet (Bozeman Fish Technology Center, Montana). Feeding regime started at approximately six times per day with 1.2 mm pellets, then decreased to two times per day over the course of the study. This was based on growth curves targeted to mimic a wild *O. mykiss* growth trajectory calculated by the Surrogate Project and OHRC personnel (Ryan Couture, OHRC Facility Manager, pers. comm.)
Monthly sampling

Monthly sampling was conducted for the duration of the study. Following anesthetization (50 mg/L MS-222 buffered with 125 mg/L NaHCO3 to pH= 7.0), 20 fish per tank were randomly sampled for fork length (FL), weight (g), tag retention (y/n) and condition factor (K). In mixed treatments, equal numbers of large and small-egg origin fish were sampled. On day 120, it was deemed necessary to pelvic fin clip the two distinct groups to continue to track large and small-egg origin fish in the mixed treatment. Using sterile surgical scissors, the left pelvic fin was clipped for large-egg origin fish and right pelvic fin was clipped for small-egg origin fish under anesthesia as described above. All fish were clipped regardless if they resided in a homogenous or mixed treatment.

Results

Egg Morphology

We observed a significant difference between the largest and smallest eggs within each of the seven individual females spawned. The egg diameter variation was observed via a photograph of a subset of each female's eggs using the program tpsDig (Rohlf, 2010). By calculating the diameter of 50 eggs within each clutch, we observed that there were significantly larger and smaller diameter eggs within each of the seven *O. mykiss* females spawned for the study. Next, by weighing 50 eggs from each female we were able to quantify the variation in egg weight to calculate the upper and lower quartiles for each female.
From these weights, the heaviest and lightest quartile for each female was calculated (Table 1).

**Growth**

Growth was examined among the three treatments conducted in duplicate (two small-egg origin tanks, two large-egg origin tanks, and two mixed (50% large-egg origin, 50% small-egg origin) tanks (Figure 2). Over the course of this study, a near normal distribution of fork lengths was observed. In analyses, the data were log-transformed to achieve a more normal distribution of this size data before statistical analyses were conducted.

A pairwise comparison allowed us to test for any differences in fork length during the study in relation to egg size. The model, with length as the response to treatment and date as fixed effects, an interaction variable of treatment by date, and a random effect for tank, allowed for an analysis of co-variance (ANCOVA) to compare the interaction of date and treatment while allowing the slopes to vary over time. Fish in the small-egg origin tanks grew to a significantly greater mean length than fish in the large-egg origin tanks ($F_{(1,94.66)}=0.008$ $p = 0.006$) (Figure 6). At ponding, the large-egg origin fish were slightly (48.2 cm) but not significantly longer in fork length than small-egg origin fish (47.3 cm). Small-egg origin fish from the uniform treatment grew longer than the large-egg origin fish from their uniform rearing treatment ($F_{(1,242.3)}=0.033$ $p = 0.06$) (Figure 4). Between month three and four (October and November 2014), the small-egg origin fish length, on average, surpassed the large-egg origin length. This trend
was maintained through day 213 (March 2015) when the project was terminated (Figure 7).

A second model (length ~ treatment + mixed + date) allowed us to test for a nested effect of rearing group (uniform vs. mixed). At each sampling event, both the large and small-egg origin fish from the mixed rearing group had longer fork length than their uniformly reared counterparts as well as small-egg origin fish having a longer fork length in either treatment at any given sampling date. This trend, although high in variation, persisted across the entire study. Figures 8 and 9 show one sampling date (Day 212) as an example of these trends.

Discussion

As I predicted, egg size varies both among and within female steelhead. Also, as I predicted, the growth of individual fish varies inversely with the size of the egg from which they originate. My study confirmed that there is significant variation in egg size within individual female natural-origin *O. mykiss*. In all cases, within female variation in egg size was significant in this study. Fish originating from the smallest eggs per female had associated faster growth than fish from the largest eggs per female within the first 8 months’ post-hatching. Fish from the smallest eggs had longer final body lengths than fish from the largest eggs. Finally, fish reared in a mixed treatment grew longer than fish reared in uniform groups, regardless of whether they originated from small or large eggs to begin.
Although we did not explore the mechanism for this phenomenon, it is clear that maternal factors can be significant in the growth and health trajectory of a juvenile fish (Einum, 2003; Solberg, Skaala et al., 2013; Vijayan & Leatherland, 1988). In contrast to the male gamete, which mostly contributes to egg quality through its DNA, the female gamete includes factors such as maternal mRNA, hormones, and yolk proteins that are later utilized to support embryonic development (Brooks, Tyler et al., 1997; Leatherland, Lin et al., 1989). Because the female gamete is contributing from the time the egg is released from the ovary at ovulation, through spawning, and into hatching, there is often difficulty in estimating/measuring what is a good (or a bad) quality egg (Wargelius, Furmanek et al., 2015). By choosing to measure individual egg size while holding constant other environmental factors (temperature, photoperiod, diet, tank size, etc.), I was able to interpret the outcome of these maternal factors combined with the genetic or epigenetic mechanisms contributed by the males in the study as manifested through growth in the first year of life (Terry D. Beacham & Murray, 1985; Funk, Tyburczy et al., 2005).

The results of this study have implications for rearing steelhead in hatchery environments. Negative effects of domestication can occur in Pacific salmon over very few generations. Those results have raised concerns for many involved in both the recovery of wild salmonids as well as the release of hatchery fish, including hatchery managers, tribes, anglers, conservation groups, and the general public. Christie et al. (2015) demonstrated that the reproductive fitness decline due to captive rearing can occur as quickly as over one or two
generations. They also stated that if selection acts on a single trait, such rapid
effects can be explained only when selection is very strong, both in captivity and
in the wild, and when the heritability of the trait under selection is high. In the
case of egg size, this is demonstrated by the fact that hatchery rearing relaxes
natural selection, allowing fecundity selection to drive exceptionally rapid
evolution of small eggs (Fox et al. 2003). In addition, trends toward small eggs
are evident in natural populations heavily supplemented by hatcheries, even
when female body size stays the same (Heath, Heath et al., 2003). In fact, the
relationship of egg size to hatchery- or wild-origin of salmon, and the
consequences of differences in egg size within a salmon species can be very
complex, and so simple conclusions as the cause and effect relationship of egg
size and life history might be premature (T. Beacham & C. Murray, 1993;
Bromage, Jones et al., 1992; Fleming & Gross, 1990; Heath, Heath et al., 2003;
N Jonsson, Jonsson et al., 1996; Thomas P. Quinn, Vøllestad et al., 2004;
Swain, Riddell et al., 1991; J. Thorpe, Miles et al., 1984; J. Thorpe, Morgan et al.,
1983; J. E. Thorpe & Morgan, 1978). We need to keep this in mind, as attempts
are underway to rebuild the natural-origin O. mykiss population in the North
Santiam River.

Research on Arctic charr (Salvelinus alpinus) helped inform the
hypothesis for my study. S. alpinus females show considerable variability in egg
size and yolk quality (C. A. L. Leblanc, Benhaïm et al., 2011). The inverse
correlation between egg size and juvenile size persists for up to 1 year after first
feeding (Wallace & Aasjord, 1984). Embryos from smaller eggs develop faster
than those from larger eggs, suggesting that different timing of development is connected to egg size (C. A. L. Leblanc, Benhaïm et al., 2011; Valdimarsson, Skúlason et al., 2002). The mechanism responsible for that differential growth is the difference in regulation of the IGF1 gene in the young fish. I predict that the same mechanism is responsible for the faster growth of young steelhead from smaller eggs.

The fact that small-egg origin juvenile steelhead grew faster than large-egg origin fish might be considered counterintuitive to current hatchery management practices. This may be because egg quality can be highly variable and difficult to measure and is therefore not currently included in most selection programs (Bobe & Labbé, 2010). Despite this, the ability to produce large numbers of high quality eggs “on demand” is an important issue for the development of aquaculture (Wargelius, Furmanek et al., 2015). Given the high cost of hatchery rearing programs, large variations in the quantity or quality of gametes can significantly affect hatchery or aquaculture goals (Bromage, Jones et al., 1992). In the case of the Wild Surrogate Project the problem is amplified by the fact that we are working with limited natural-origin populations often listed under the Endangered Species Act of the United States in an effort to assist the recovery of wild populations that once thrived in the region.

An element to consider is the possibility of a bet-hedging tactic being employed by the female to increase the diversity of her offspring (Marshall, Bonduriansky et al., 2008). This phenomenon is observed across taxa and consists of organisms sacrificing short-term success to reduce the long-term
variance in success (Chisholm, Ellison et al., 1993). For example, winter annual plants in the Sonoran Desert adaptively delay germination in a bet-hedging strategy (Kimball, Gremer et al., 2012). In relation to salmonid egg size, it is generally expected that environmental unpredictability will cause females to hedge their bets (Koops, Hutchings et al., 2003). There are two types of bet-hedging: conservative and diversified (Einum & Fleming, 2004). Conservative bet-hedging corresponds to producing fewer but larger offspring than would be the optimum in a stable environment, while diversified bet-hedging is achieved by increasing the phenotypic variance among individual offspring to ensure that at least some of them are successful (Philippi & Seger, 1989). In my study, diversified bet-hedging would correspond to producing variable egg sizes by a single female within a clutch. If this is not the mechanism, perhaps within-clutch variation is a physiological/developmental constraint of the female’s ability to evenly allocate resources (Einum & Fleming, 2004).

Based on my results and the background knowledge of the influence of environmental factors, maternal factors, management bias (i.e. which fish are spawned by hatchery managers or aquaculture facilities), and evolutionary and genetic factors, it is clear that a large challenge still exists when it comes to recovering wild populations. Some major challenges that I identify for future research include the lack of systematic phenotyping of early embryonic success, a need for a deeper understanding of what makes a desirable egg (i.e. identification of robust and genetic molecular markers of egg quality), and most importantly, the continued pursuit to better understand potential interactions and
the consequences of the interactions between hatchery and wild salmonids (Christie, Marine et al., 2012; Wargelius, Furmanek et al., 2015).

After considering a potential difference between hatchery and wild salmonids, studies must also describe the variation within a single broodstock. There could be a nexus of cause and effect relationships among growth rate, age and size of females that influence their reproductive allocations. In general, large females have a larger body cavity with more physical space for their ovaries (Blueweiss, Fox et al., 1978). However, a given ovarian volume in that space could be occupied by a larger number of smaller eggs or a smaller number of larger eggs. Although, higher ovarian mass has been linked to migration distance, the number of eggs rather than egg size was associated with this evolution (Kinnison, Unwin et al., 2001).

Although my study was robust in that it was carried out past the point at which many egg size studies are terminated, one of the main challenges facing the broodstock used in this study is the interaction between hatchery-origin and natural-origin fish in the Willamette River basin and the fact that the fish used in this study sit somewhere in the grey area between those two groups. Another limitation to my study was that the ages of the fish sampled were not determined. We selected females of approximately equal size for my study, with the assumption that they would be of similar ages. An important next step to continue this research would be to determine if egg size is related to the growth rate, age or size of individual females. Salmonid life history theory would be the basis for predictions to be tested by such studies (Eric L Charnov & Gillooly, 2004).
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CHAPTER 3- COMPLEX STRUCTURE AND FIN QUALITY IN WILD BROODSTOCK STEELHEAD (ONCORHYNCUS MYKISS)

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Introduction

One of the main critiques of raising fish in a hatchery environment for release into the wild is that lower survival is often observed than fish of the same age that have been rearing in natural streams. The lower survival of hatchery-reared fish is often ascribed to factors such as genetic domestication and unnatural rearing environments (Hitoshi Araki, Cooper et al., 2009; M. Bradford, Berejikian et al., 2010; Rosengren, Kvingedal et al., 2016). Those critiques often include suggestions for manipulating hatchery rearing conditions to produce higher survival of fish once they are released into the wild. Some common suggestions for conditions that might be manipulated in a hatchery environment include the density of fish per tank, type of diet or feeding regime, temperature, water flow, and structure or “enrichment” added to the rearing tanks (BA Berejikian, Tezak et al., 2001; C. Brown, T. Davidson et al., 2003; Hyvärinen & Rodewald, 2013; Marchetti & Nevitt, 2003; Näslund, Rosengren et al., 2013; Rodewald, Hyvärinen et al., 2011). These manipulations are most often aimed at improving fish quality whether it be for later harvest and consumption or release into the wild to supplement a natural-origin population.

The goal of my study as part of the larger Wild Fishes Surrogate Project was to raise offspring from natural-origin steelhead (Oncorhynchus mykiss) broodstock to mimic a wild fish growth trajectory for both fin morphology and growth, compared to wild conspecifics in their native Willamette River basin. This was accomplished by rearing fish from emergence to release size with or without complex structure present with the prediction that fish reared with complex
structure would both better mimic a wild growth trajectory and have better fin quality at age of release.

The Wild Fishes Surrogate Project is a collaborative project between Oregon State University (OSU), Oregon Department of Fish and Wildlife (ODFW), and the United States Army Corps of Engineers (USACOE). The Project’s mission is to develop rearing strategies to produce wild juvenile surrogate fish for use by researchers in dam passage studies (Noakes, Schreck et al., 2013). This is accomplished by providing juvenile Chinook salmon and steelhead trout of hatchery origin to researchers to serve as surrogates for wild fish where the capture and tagging of wild fish is not possible. Wild fish are not available because populations of these species in the Willamette River are threatened or at risk of extinction (Monzyk, Romer et al., 2012). By monitoring the behavior of these surrogate fish, we will gain a better understanding of the responses of fish to reservoirs and dam passage than we would if typical hatchery fish were used in field studies (Billman, Whitman et al., 2014).

The North Santiam River provided the *O. mykiss* broodstock for my study. The North Santiam River is a 92-mile (148 km) tributary of the Santiam River in western Oregon, USA. It drains 766 square miles (1,980 km²) of the Cascade Range on the eastern side of the Willamette Valley, east of the state’s capital city of Salem. Blocked by Big Cliff Dam near Mill City, OR, the lower North Santiam River supports spring Chinook salmon and summer steelhead populations. In the reaches upstream of the dam, the river is managed mainly as a stocked-trout (non-migratory *Oncorhynchus mykiss*) fishery. Near the stream’s source at
Santiam Lake in the Mount Jefferson Wilderness, native cutthroat trout 
(*Oncorhynchus clarkii*), rainbow trout, and brook trout (*Salvelinus fontinalis*) are also found (USACOE, 2011). There are two dams present, Big Cliff and Detroit Dams at river mile 46 and 49 (km 74 and 79), respectively, which produce a combined output of almost 120,000 kW of hydroelectricity. Just downstream of these dams is Minto Fish Collection Facility that is owned and operated by the USACOE. That state-of-the-art facility was opened in 2013 in response to the Biological Opinion (BiOp) in the parlance of the Endangered Species Act, which called for a new “safe and effective” fish collection system at Minto (ODFW, 2011).

Despite these and other state-of-the-art facilities, a number of populations of wild salmonids in the Willamette River Basin are listed as either threatened or endangered under the federal Endangered Species Act (Region & Thom, 2010). In part, this has led to the Wild Surrogate Rearing Project in which we closely monitor many early life history parameters, but here focus on the growth and the fin quality of juvenile steelhead. Growth is important to track because we are not rearing fish on a typical hatchery growth trajectory, which often appears linear. Production hatcheries often conduct their operations to produce maximal growth rates in young fish so that they are of a size comparable to migrating conspecific smolts within 12 months of age (N. F. Thompson, Leblanc et al., 2015). Instead, in our Wild Surrogate Project we aim to mimic a more wild-like growth trajectory by either allowing the fish to be reared in the natural Fall Creek water temperature regime or, when necessary, controlling the temperature of the water
to regulate growth rate. Growth is also directly controlled by our experimental
diet and the method of feeding. Our experimental wild fish grower diet more
closely matches lipid and protein levels found in a natural stream diet than
commercially available feed. In addition, we feed on an adaptive schedule that is
outlined explicitly in the Methods.

In addition to obvious growth patterns, a second, easily quantified,
identifier of overall fish health is fin quality (Abbott & Dill, 1985; Barry Berejikian,
2005; T Ellis, Hoyle et al., 2009; Kavanagh & Olson, 2014). In fact, eroded fins
are often accepted as obvious evidence of hatchery origin of salmon taken in the
wild. Fin erosion in the hatchery environment is well-known and may be a factor
in reducing fish survival (Bosakowski & Wagner, 1995; Latremouille, 2003). Poor
fin quality can lead to impaired swimming which can impact foraging or migration
ability, and reduced overall health resulting in higher disease susceptibility
(Schneider & Nicholson, 1980). Measuring fin quality is a noninvasive method
for quickly assessing fish physical fitness as well as an indicator of hatchery-
origin fish in a natural environment since they often look noticeably different than
stream or reservoir reared salmonids. This has been demonstrated in an Atlantic
salmon (Salmo salar) comparison between stream-reared young, smolts from the
main stem of a river and hatchery reared parr. In all cases, the fish reared in the
stream environment had less fin degradation than hatchery reared parr that
degraded continually over time (Pelas & McCormick, 2003).

The structural complexity of the rearing environment (mainly to study
behavior and the effects of growth patterns) has been studied extensively (BA
with some studies also investigating the effects of structural complexity on fin damage over a longer time period and in intense rearing systems (Berejikian, 2005; Persson & Alanärä, 2014). In addition, the use of complex environment in production-style tanks has been shown to improve feeding rates of juvenile salmon on natural prey (Rodewald, Hyvärinen et al., 2011).

Ultimately, the rearing environment may have a large impact on survival. In the Tornionjoki River in Canada, Atlantic salmon (Salmo salar) that were reared with what the authors deemed enriched methods had a twofold increase in survival (~38%) compared with smolts that had been reared in a standard hatchery environment (Hyvärinen & Rodewald, 2013). This example can be directly compared to our Wild Fishes Surrogate Project where, although our surrogate fish are not identical to wild fish, they are on average much more like their wild, or natural-origin, counterparts than they are to the conventionally raised hatchery salmon in Oregon (Billman, Whitman et al., 2014). By tracking the growth and changes in fin quality of our Wild Surrogate O. mykiss from emergence to release age, we could assess the potential effect of the presence of complex structure in the experimental hatchery environment.

Methods

My study is part of a multi-agency project primarily funded by the USACOE designed to examine the effects of dams on the early life history
survival of juvenile salmonids produced from wild-broodstock adults from the North Santiam River. Spawning of wild *O. mykiss* broodstock occurred in April 2015 at the Minto Fish Collection Facility located on the north bank of the North Santiam River, 4 miles (6 km) downstream of Big Cliff Dam and 7 miles (9 km) downstream of Detroit Dam (USACOE, 2011).

**Broodstock Acquisition and Incubation**

See Chapter 2 for a detailed description of broodstock acquisition.

**Rearing of Brood Year 2014**

Once at the OHRC, each matrix cross was kept in a separate mesh basket to incubate in Heath trays. This prevented individual female’s embryos from mixing. All embryos were incubated in temperatures that mimic the North Santiam River’s cool thermal regime (approximately 6.5-7°C). Shocking and picking of dead embryos occurred in late May 2014 at which time the developing embryos for this study were sorted.

Upon emergence, fish were reared in two indoor troughs until being ponded in 2-meter diameter outdoor tanks, following a low-density protocol (200 fish per tank) typical of experimental hatcheries (Rand, Berejikian et al., 2012). The experiment was conducted in triplicate until the last 2 months of the study at which time it was reduced to duplicate due to facility constraints.

Fish were hand fed the Wild Surrogate Project low-lipid (8-9%) wild steelhead grower diet (Bozeman Fish Technology Center, Montana). The
feeding regime started at approximately 6 times per day with 1.2 mm pellets, then decreased to two times per day over the course of the study. Fish were fed an amount of feed each day calculated to produce our Wild Surrogate growth pattern, based on growth curves targeted to mimic a wild *O. mykiss* growth trajectory calculated by the Wild Surrogate Project and OHRC personnel (Couture, 2017).

**Adaptive feeding regime**

Feed was calculated and weighed in relation to mean body size and desired growth trajectory once per week with fish being presented food to apparent satiation each day thereafter for 7 days in total. Once the total amount of feed allocated for a given week was eaten by the fish, no more food was provided for the rest of that week. The food was replenished once per week per tank. Typically all food was gone within 3 to 5 days, resulting in the possibility of less or no food being available at certain points in the feeding schedule to mimic a wild feeding regime. This adaptive feeding is based not only on the size of the fish but also water conditions (turbidity, flow, etc.), water temperature, and weather. This adaptive feeding regime significantly reduces the bimodal size distribution that is commonly found in hatchery-reared salmonids (J. Thorpe, 1977).

**Artificial In-Tank Structure and Monthly Sampling**

All fish were reared with structure for 2 months following emergence in indoor troughs at the OHRC before being ponded outdoors in 2-meter diameter
outdoor tanks with camouflage covers for the remaining 6 months of the study. This structure was constructed of 1.5-inch diameter PVC piping and black plastic sheets cut into approximately 1.5 inch strips. One long spine with two centered and slightly shorter perpendicular arms was laid horizontally (to represent log habitat) or left vertical (stump/root habitat). Holes in the PVC pipe that rested on the bottom of the tank allowed water to fill the pipe so that the structure remained stationary in all sizes of tanks at the OHRC (Figure 10, Figure 11). This design is scalable and can be constructed with varying dimensions and placed in tanks of varying dimensions and/or fish densities. Water flowed in the tanks at the recommended velocity of approximately 1-1.5 times the mean length of the fish being held (Farlinger & Beamish, 1977). Water was single pass, unfiltered Fall Creek Water, at ambient temperature and dissolved oxygen conditions (D. L. G. Noakes & C. Corrarino, 2010).

Monthly sampling was conducted for the duration of the study (30 fish/tank), where, following anesthetization (50 mg/L MS-222 buffered with 125 mg/L NaHCO3 to pH= 7.0), fish were randomly sampled and measured for fork length (FL) to the nearest millimeter and weight was recorded (nearest 100\textsuperscript{th}/g) which allowed for Fulton’s condition factor to be calculated (K). In tanks containing structure, we found it necessary to remove the structure to randomly subsample the juvenile fish. The structure was replaced immediately following sampling of the fish.

*Morphometrics*
Fin and body morphology were compared between the treatments using landmark-based geometric morphometric analysis (Kristjánsson, Skúlason et al., 2012). Digital photographs provided the pictures for landmarks (Pentax Optio WG-2 GPS, 16Mp resolution). Quantification of dorsal fin and caudal fin shapes was conducted using three and five landmarks, respectively, and both were digitized using tpsDig (Rohlf, 2010). Landmarks fell on the snout, origin of dorsal fin, end of first full dorsal fin ray, insertion of dorsal fin, base of caudal fin, first ray of dorsal lobe of the caudal fin, middle fork of caudal fin, first ray of anal lobe of caudal fin and posterior caudal fin connection point (Figure 12). Specifically marking the fork length in the morphometric software allows for precise comparison of the length of certain fins to fish fork length.

Statistics

All statistical analyses were conducted using the statistical software R (Team, 2013). After checking for normal distribution (Bartlett’s Test) and equal variance of the data. Preliminary growth between the treatments was analyzed using a linear regression (lmer) analysis of variance model to compare fish lengths between treatments. This allowed for each sampling event to be tested as well as random effects, such as tanks.

To assess fin quality, log-transformed data were used in a separate linear regression analyses (lm) to compare the dorsal and caudal fin lengths between treatments. The body length of each individual fish was included as a co-variate to account for allometric growth (fin length ratio = fin length/body length). Due to
non-significance of the effect of body size, the interaction term between treatment and body length was eliminated from the final model. The differences in dorsal fin length was compared both across the entirety of the study as well as at each sampling date.

Both length of the longest ray of the caudal fin and asymmetry in the entire caudal fin between treatments was compared using a linear regression as a potential indicator of different rates of erosion between treatments.

To show that the fin deterioration was not purely a size-driven result, the origins of the dorsal fins of fish allocated into ‘small’ and ‘large’ groups (calculated using the top and bottom quartiles of the size distribution for each treatment) were compared using a linear regression (lm) analysis of variance. In all cases we accepted $p < 0.05$ as indicative of statistical significance.

**Results**

Fish reared both with and without complex structure were the same mean fork length at the conclusion of the study (Table 2). Slopes and intercepts were not significantly different from each other over the course of the 9-month study ($p > 0.5$).

After removing a body length by treatment interaction ($p=0.85$), a linear regression comparing the length of the first dorsal fin ray showed that fish raised with complex structure had significantly longer dorsal fins at the final sampling event (April 2015) ($F(2,52)=2.21$, $p=0.042$), although anterior dorsal fin length did not vary between treatments over time in general ($F(2,318)=53.91$, $p=0.845$).
The dorsal lobe of the caudal fin for fish raised in the structure treatment was, on average, longer than the dorsal lobe of the caudal fin for fish raised without structure (F (4,316)=1042, p=0.008) The caudal fin in relation to body length varied between treatments, indicating asymmetry (F (4,316)=2.441, p=0.047).

To show that dorsal fin deterioration was not being driven by size, small and large fish from both treatments were compared using linear mixed effects model (ANOVA: F (2,27)=1.248, p=0.28). This could imply that the decrease in fin ratio over time is due more to the allometric growth of the fish, i.e. the ratio would be decreasing over time because the fish are growing longer faster than the dorsal fin is increasing in size and that the data are not independent.

Discussion

Fish reared with complex structure grew at the same rate as fish without complex structure over the course of this study. Fish reared with complex structure had longer anterior dorsal fins at the final sampling event, but dorsal fin length did not differ significantly at other points in the study. The dorsal lobe of the caudal fin was longer in fish reared with structure, and the caudal fin in relation to body length ratio was found to vary between treatments, indicating asymmetry. Fins deteriorated at the same rate between small and large fish in both treatments, which could show an effect of allometric growth. This makes sense since it is not expected that dorsal and caudal fins would grow isometric to body length in a maturing salmonid.
The differences observed support my hypotheses that fish reared in the presence of complex structure would, a) better mimic a natural growth trajectory than a fish reared in an average hatchery environment and b) have a higher fin quality (less fin degradation) than fish raised in bare tanks, at age of release.

The presence of a growth curve similar to that of a natural-origin juvenile salmonid, is evidence that one or more of the experimental variables employed by the Wild Surrogate Fishes Project influence fish growth as measured by fork length. As a reminder, among these variables are an experimental diet, varied feeding regime, fish reared at a density below conservation hatchery standards, water temperatures that follow seasonal variations of the source at Fall Creek and Carnes Creek, and fish spawned from natural-origin broodstock in the North Santiam River.

Temperature can drastically influence the growth rate of ecothermic species such as *Oncorhynchus mykiss*, especially in a controlled hatchery environment. Temperature in production hatcheries has long been controlled by managers to achieve maximum growth in a given time frame. This has been calculated in relation to temperature and food rations for steelhead (Parker & Larkin, 1959), coho salmon (*O. kisutch*) (Stauffer, 1973), and brown trout (Elliott, 1975). Although the formula differed for each species, all of these models aimed for a linear growth pattern to maximize growth in a controlled environment (Iwama & Tautz, 1981).

By allowing growth rates to vary, we rear Wild Surrogate Project fish to better mimic their natural-origin counterparts rearing in streams. Growth rate
changes based on many things, including physiological changes related to life history stages (i.e. smoltification), seasonal temperature fluctuations, and prey availability, to name a few (Gale, Hill et al., 2004; J. N. Thompson & Beauchamp, 2016). Fish that are exposed to variation in their environment, even when they are from the same broodstock, have been shown have equal fitness on average despite displaying non-uniform growth rates (Mangel & Stamps, 2001).

Therefore, we do not consider a non-linear growth curve in the hatchery environment a detriment to the size at which the fish will be released to our collaborators, but rather an indicator that the Surrogate fish are modulating their growth as a natural-origin salmonid might.

In addition to growth, fin quality is easily quantified as an indicator of overall fish health (T Ellis, Hoyle et al., 2009). Fin erosion in the hatchery environment is a well-known effect of fish rearing density (North, Turnbull et al., 2006) and may be a factor in reducing fish survival (Bosakowski & Wagner, 1995; Latremouille, 2003). Individually, fin erosion has received attention in European aquaculture where it represents injury to live tissue containing blood vessels, nerves, and probably nociceptors (Tim Ellis, Oidtmann et al., 2008). The differences in dorsal and dorsal lobe of the caudal fin degradation, although significantly less in the complex structure treatment, were still lower than has observed with _O. mykiss_ reared in a conventional hatchery setting when compared to similar studies (Barry Berejikian, 2005). Fish diet, rearing density, the presence or absence of complex structure, and genetics could all play a part in fin quality of juvenile salmonids in a hatchery environment.
Fish diet (feeding method and nutrition) can play a direct role in fin quality through nutrition and water quality. Barrows and Lellis thoroughly showed that the dietary agent contributing to dorsal fin erosion in *O. mykiss* is present in the protein or mineral fraction as opposed to the ether-extractable (lipid) fraction of the diet (1999). A stark difference in juvenile diets is that a natural-origin stream fish would depend mostly on invertebrates, while commercial diets used in production hatcheries are often based on protein from marine fishes, which can contain significant quantities of vertebrate hormones (Feist & Schreck, 1990).

Likely influencing both growth and fin quality, is the rearing density of steelhead. Rearing *O. mykiss* at higher density has been shown to reduce food intake, food conversion efficiency, growth rate, and body, liver, and fin condition indices (T Ellis, North et al., 2002). Contrarily, maintaining *O. mykiss* at too low a density can also be detrimental to welfare resulting in reduced foraging ability and even mortality through excessive aggressive behavior (T Ellis, North et al., 2002; J. W. A. Grant, 1993). We aimed to avoid possible negative outcomes by rearing fish at densities below both conventional and conservation hatchery standards, but high enough to avoid reduced growth and increased aggression. Rearing density is also important post hatchery release. Rearing density for brown trout (*Salmo trutta*) has been shown to directly influence survival in a natural stream, with brown trout reared at a natural density (versus conventional hatchery density and half of conventional hatchery density) being twice as likely to survive in a natural stream after release (Brockmark & Johnsson, 2010). Based on studies like this, we hope to increase the chance of survival for the *O.*
*mykiss* smolts released as part of the Wild Surrogate Fishes Project by rearing them at favorable densities for this life-history stage.

Fish reared on complex structure showed less dorsal and anterior caudal fin degradation than fish reared in bare hatchery tanks. This dorsal fin pattern has been observed in juvenile steelhead reared in conventional hatchery tanks, enriched tanks, and a natural stream but all were reared at a higher density than in our study (Barry Berejikian, 2005). Not only is there evidence that complex structure in the rearing environment may improve fin quality, there is also evidence that it may improve feeding rates of juvenile salmon on natural prey in salmonid species (Rodewald, Hyvärinen et al., 2011).

In this study, because it is unlikely that fin nipping or biting were due to the density at which the fish were reared, it is possible that foraging competition was a factor. In tanks with complex structure, fish may have been better able to set up territories or avoid direct competition for food, although this was not measured. It is unclear if this is applicable because although some studies that have shown that, after controlling for density, territory size of juvenile steelhead trout changes in response to changing food levels and competitors (Keeley, 2000), others have shown relatively little change in *O. mykiss* territories in response to the same factors (Toobaie & Grant, 2013). Although it may not be clear to us why fin nipping occurred at a measurable level, it occurred more often in the tanks lacking complex structure. Based on seminal work by Abbott and Dill, we know that in juvenile steelhead trout the dorsal fin incurs the greatest damage and damage is most commonly observed in hatchery-reared salmonids. They also
reported that, in nonreciprocal bouts, aims were directed at the dorsal fin, central body, and caudal fin, while nip contact was biased toward the caudal fin. In reciprocal bouts, both aims and nips were concentrated on the dorsal fin and anterior portions of the body (1985). Therefore, our results are consistent with previous studies of *O. mykiss*.

Although less is known about how genetics may impact growth and fin quality in the hatchery environment, hatchery steelhead juveniles raised in tanks at low density and low food rations have been associated with elevated agonistic behavior compared to wild steelhead juveniles raised in similar conditions (B. A. Berejikian, Mathews et al., 1996; "<Berejikian-1995-The effects of hatchery and wi.pdf>,") which leads to fin nipping and degradation. Some studies have also successfully compared summer steelhead growth between hatchery x hatchery (HH), hatchery x wild (HW), and wild x wild (WW) in both a hatchery pond and sections of streams. In streams, WW fish had the highest survival and HW fish had the highest growth rates while, in the hatchery pond, HH had the highest survival and growth rates (Reisenbichler & McIntyre, 1977). This may show adaptation to novel environments outside of the natural stream within a few generations which has been observed in as little as one generation of hatchery spawning (N. F. Thompson & Blouin, 2015).

Considering the known variables in my study (diet, temperature, rearing density, complex structure), we can conclude that the growth curve observed is likely due to the temperature regime and experimental diet that all fish were subjected to. In the case of fin quality, we conclude that the difference between
the groups is not due to direct tank abrasion or diet deficiencies, but more likely direct fin nipping or displays of aggression between the steelhead themselves and the presence or absence of complex structure in the tank.

Ultimately, the rearing environment may have a large impact on survival after release. While we acknowledge the lack of a direct conventional hatchery comparison, both this study and the best available literature point to fish reared without complex structure having dorsal and anterior caudal fins that more closely resemble those of conventional hatchery management, while fish reared with complex structure have fins that more resemble natural-origin fishes (Bosakowski & Wagner, 1994). This can be important not only to increase the survivability of hatchery-raised fish to be stocked in the wild, but also to increase the aesthetic value of stocked fish to the angler and public fish consumer (Bosakowski & Wagner, 1995). Most importantly and on a larger scale, the goal of our project is to work towards the recovery of the federally endangered run on the North Santiam River of steelhead trout (Noakes, Schreck et al., 2013).
Literature Cited


CHAPTER 4- THE EFFECT OF COMPLEX STRUCTURE ON THE GROWTH AND MOVEMENT OF JUVENILE ONCORHYNCLUS MYKISSL

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Introduction

As a juvenile anadromous salmonid migrates downstream from its freshwater spawning grounds to the ocean, individuals only have a 30% chance of survival in the process (Nina Jonsson, Jonsson et al., 2003; Saloniemi, Jokikokko et al., 2004; N. F. Thompson & Blouin, 2015). This represents a significant hurdle for a juvenile salmonid to clear on its road to becoming an adult (Monzyk, Romer et al., 2012). Key factors in this migration include foraging ability, successful smoltification (the physiological changes required for the successful transition to salt water), and predator avoidance during movement downstream (Baynes, Howell et al., 1994; Brown & Laland, 2001). These challenges are amplified by the fact that, in the Pacific Northwest and elsewhere, humans have often constructed one or more dams and reservoirs to the freshwater rivers salmonids use, resulting in decreased survival rates during downstream migration (Hostetter, Evans et al., 2012; B. Jonsson & Jonsson, 2009)

Complicating these challenges is the well-supported fact that hatchery-origin and natural-origin salmonid smolts do not survive at equal rates during their migration to the ocean (Brown & Laland, 2001; Zydlewski, Foot et al., 2003). On a world-wide basis, around 5 billion hatchery reared salmon are released annually but fewer than 5% survive to adulthood (McNeil, 1991). It can be noted that, in salmon, there is some agreement that if a smolt can survive the first 3 weeks of life in the wild then its chances of survival are substantially improved (Brown & Laland, 2001), and in the hatchery environment there is
usually over a 98% survival rate through this critical period. In most watersheds hatchery-origin and wild-origin fish coexist which results in competition for resources and habitat. In the last 25 years, it has become clear that conventional hatchery efforts have not always been able to produce fish that can contribute to or supplement the wild populations that once flourished in the USA (Amoroso, Tillotson et al., 2017; Nehlsen, Williams et al., 1991).

In an effort to better understand hatchery-origin smolt survival, tagging studies have been conducted with salmonids reared in conventional hatchery environments including steelhead (*Oncorhynchus mykiss*) in the North Santiam River (Romer, Leblanc et al., 2013). However, differences in morphology (Carl & Healey, 1984; Kihslinger & Nevitt, 2006; Tiffan & Connor, 2011; Wessel, Smoker et al., 2006), physiological responses to stress, disease susceptibility (Hedrick, Adkison et al., 1998; Salonius & Iwama, 1993), predator avoidance (Fritts, Scott et al., 2007), and territorial behaviors (Metcalf, Valdimarsson et al., 2003) as well as fish development (Hansen & Jonsson, 1991; Shrimpton, Bernier et al., 1994) and survival (Chittenden, Biagi et al., 2010; Fritts, Scott et al., 2007; Magnusson & Hilborn, 2003) between hatchery- and naturally-reared salmonids have been well documented. Hatchery-origin steelhead differ in a number of ways from wild fish at juvenile life history stages (Hill, Zydlewski et al., 2006; Kostow, 2004; McLean, Bentzen et al., 2004). Rearing practices can alter appearance (fin condition) and performance (fitness) of steelhead. It is likely that many of these reported differences influence migration behavior of hatchery fish, potentially confounding estimates of dam passage survival. We strive to
understand how the differences might either affect interpretation of results from dam passage studies using hatchery reared juveniles or how hatchery rearing procedures might be altered to minimize the differences between hatchery- and natural-origin fish.

As a consequence of known differences between hatchery fish and natural-origin or “wild” fish, the emphasis of hatchery operations has changed from measures of quantities of fish produced to the quality of those fish. Specifically, rearing parameters that may accomplish this include enhanced feeding opportunities, low human interaction, low fish density, and structure (Zydlewski, Foott et al., 2003). Targeted projects have been implemented to produce higher quality fish in a hatchery environment by trying to mimic those conditions experienced by wild fish. The assumption is that fish reared in this manner will have physiological and behavioral characteristics more similar to those observed in the wild, or, as the Northwest Power Planning Council clearly stated as early as 1999, fish that are “behaviorally, morphologically and physiologically indistinguishable from their wild counterparts” (Zydlewski & Johnson, 2002). It should be noted here that my thesis, guided by this assumption, contributes to a greater body of ongoing work being conducted by the Wild Fishes Surrogate Project at Oregon State University (Noakes, Schreck et al., 2013). The research detailed in this chapter contributed to research on the early and juvenile life history stages of steelhead trout from the North Santiam River, reared from spawning to smolt stages at the Oregon Hatchery Research Center (OHRC). Ideally, by considering the differences between hatchery-origin
and wild-origin salmonids (morphological, behavioral, genetic, etc.), hatcheries could produce juveniles that have the same physiological and behavioral characteristics as their wild counterparts, with the goal of improving survival of released smolts (Brown & Laland, 2001).

It is challenging for a hatchery to produce a healthy wild-like fish. In addition to successful smoltification, any salmonid that is released from a hatchery environment must have, or quickly acquire, appropriate migratory, feeding and anti-predator behaviors (Lund & Heggberget, 1992). Foraging is perhaps most imperative to survival, and laboratory experiments indicate that hatchery-reared salmonids such as pink salmon (O. gorbuscha), rainbow and brown trout all improve feeding behavior with learning. In Atlantic salmon, (Stradmeyer & Thorpe, 1987) showed that the fish changed from pellet food to wild prey over 16 trials in just 90 minutes. Additionally, Mesa (1991) added both hatchery and wild cutthroat trout (O. clarkii) that into an experimental stream and allowed them to feed on live prey. Both groups fed at similar rates, but hatchery fish were more aggressive and were slower to return to their base or refuge, which could lead to predator exposure when released into the wild (Mesa, 1991).

In addition to foraging, the ability to seek shelter in the presence of a predator is key to survival. This is especially important in a novel environment, such as after an abrupt hatchery release or as any salmonid migrates downstream for the first time as a juvenile. Higher mortality rates experienced by hatchery-reared fish may be due to fish having had no prior experience of predation and are subsequently easily preyed upon (Brown & Laland, 2001).
Proper responses to stimuli that represent or signal the presence of danger may allow an animal to avoid predation or other forms of danger in nature. More significantly, the ability of the animal to react appropriately may represent a significant fitness component (Ahmed, Seguin et al., 2011).

One thought in fisheries management is that hatchery-reared fish, when reared with complex structure may exhibit behavior that will allow for predator avoidance upon release to a natural system. The structural complexity of the rearing environment (mainly to study behavior and the effects of growth patterns) has been studied extensively (BA Berejikian, Tezak et al., 2001; B. A. Berejikian, Tezak et al., 2000; Braithwaite & Salvanes, 2005; Brockmark, Neregård et al., 2007; Zydlewski, Foot et al., 2003). The use of complex structure in the hatchery environment is sometimes viewed as a way for an individual fish to set up a territory and possibly increase its spatial awareness (Braithwaite & Salvanes, 2008). This may be beneficial in a stream ecosystem that is inherently complex, especially compared to a conventional hatchery environment. Steelhead fry reared in an enriched hatchery environment, including submerged structures, achieved significantly greater social dominance ranks than fry reared in a conventional hatchery environment (BA Berejikian, Tezak et al., 2001). In another example, juvenile brown trout from natural populations reacted to the presence of piscivorous brown trout by increasing the use of refuges (Alvarez & Nicieza, 2003). The ability of an individual to set up a territory and understand the surrounding environment may result in a greater chance of survival post hatchery release.
Anti-predator behavior is, more often than not, an energetically costly exercise because it must be traded off against other activities (Wishingrad, Chivers et al., 2014). Separation from selection happening in nature, due to domestication in the hatchery for many generations (on an evolutionary time-scale), is more likely to result in large-scale loss of responses compared with separation from a native population during an individual’s lifetime (Brown & Laland, 2001). This idea was demonstrated by (B. A. Berejikian, 1995) when his group reared two populations of steelhead in the laboratory, one from a long term (about 20 year) hatchery stock and one from wild fish, before testing the effect of predator observance on survival. After approximately 24 h, half of the fish had been allowed to observe a predator eating sacrificial conspecifics, all fish were allowed to interact with the predator and the number of surviving fish were recorded. A higher proportion of hatchery-reared fish were eaten compared to wild fish, but training improved the survivorship of both populations. Perhaps, by adding complex structure to the hatchery environment, losses such as this can start to be mitigated. By increasing the rate of novel experiences in the rearing environment, including the provision of anti-predator and foraging training, it may be possible to overcome the differences in behavior between hatchery-origin and wild-origin fish before release into the wild (Wiley, Whaley et al., 1993).

2014 Study

The stock of *O. mykiss* used for this study were wild adult fish returning to the North Santiam River in Oregon, USA in the Spring of 2014. This area is of
particular importance because it is one of the few remaining historical habitats for winter-run steelhead within the steelhead trout Evolutionarily Significant Unit (ESU) that does not currently contain winter steelhead. The Upper Willamette River Conservation and Recovery Plan for Chinook Salmon and Steelhead (ODFW, 2011) emphasizes the need for successful reintroduction of steelhead above high-head dams because the majority of spawning habitat for anadromous salmonids is upstream of these projects. By using the offspring of wild fish we may determine which alternative methods of hatchery rearing to produce wild-fish “surrogates”, as well as dam construction and operation, will best increase the survival of wild runs in coming decades (Rand, Goslin et al., 2012).

Our goal in this study was to compare possible foraging and behavioral differences exhibited by juvenile steelhead trout (*Oncorhynchus mykiss*) from natural-origin parents, raised either with or without complex structure present in hatchery tanks at the Oregon Hatchery Research Center. Assessments included whether or not there was a difference in either foraging ability or predator-avoidance behavior between juvenile steelhead raised either with or without complex structure in their outdoor hatchery tank environment. These results will contribute to current research on whether or not it is possible to produce a “wild-like” fish in a modified hatchery environment.

To accomplish this goal, we compared fish reared in tanks with artificial structure from the ponding stage by measuring: 1) latency to respond to a mock predator, 2) duration of the response, and 3) modification of response after being exposed to a predator threat more than once. Finally, we compared behavior of
the experimentally – reared steelhead to the behavior of fish reared without artificial in-tank structure. These targets allowed us to assess whether complex structure being present in a hatchery tank caused differences in behavior during juvenile fish development.

Based on previous work studying sub-yearling behavior of *O. mykiss*, we predict that we will see more use of structure by fish reared on in-tank structure at the end of a behavioral trial. In addition, as fish are exposed more than once to a predator, they will become more likely to seek cover, which would translate to a modification of behavior and avoiding predation in the wild (B. A. Berejikian, 1995).

Therefore, the hypotheses were that fish raised on structure would be better able to determine the potential risk of foraging and that fish raised on structure would be slower to stop seeking cover and resume foraging in the presence of a predator. Additionally, foraging ability would improve over the course of the behavioral trial for each individual trial with fish raised with complex structure having the highest amount of foraging success.

**Methods**

This study is part of a multi-agency project majorly funded by the USACOE, with an overarching goal of examining the effects of dams and dam passage efficiency of juvenile salmonids. Specifically for *O. mykiss*, juveniles from wild-broodstock adults from the North Santiam River are used by this study to produce wild-like fish in a hatchery setting (wild fish surrogates). Spawning of
wild O. *mykiss* broodstock occurred in April 2015 at the Minto Fish Collection Facility located on the north bank of the North Santiam River, 4 miles (6 km) downstream of Big Cliff Dam and 7 miles (11 km) downstream of Detroit Dam (USACOE, 2011).

**Brood Stock Acquisition**

See Chapter 2 for a detailed description of broodstock acquisition.

**Adaptive feeding regime**

See Chapter 3 for a detailed description of the adaptive feeding regime used in this study.

**Artificial In-Tank Structure and Monthly Sampling**

See Chapter 3 for a detailed description of artificial in-tank structure and monthly sampling protocols.

**Behavioral Trials**

Behavioral trials took place April 6-May 7, 2015 at the OHRC. Outdoor rearing tanks were randomly subsampled for up to 64 - 68, size-matched, sub-yearling steelhead each from the structure treatment and control treatment. Due to technological complications (e.g. camera battery life and LED lighting), 44 fish from the structure treatment and 51 fish from the control treatment yielded usable results.
Behavioral tanks

The 10 behavioral observation tanks in the OHRC wet lab are 90 cm wide, 6 cm deep and 60 cm tall. All sides of the behavioral tanks are blue in color except for the back, which is white, and the front, which is clear glass. The white background in each tank is removable so we used a black waterproof crayon to divide the wall into four quadrants to use for later spatial movement measurements (Figure 13). We cut clear plexiglass plastic covers with holes only for feeding and simulated predator entry to prevent fish from escaping. In addition, each tank contained a miniature version of the structure that was used in the outdoor structure treatment tanks (Figure 14). This structure was placed in the bottom front left section (when facing the setup) of the tank. Above each tank is a system of adjustable LED lights capable of displaying natural, white, blue, or red light (60 watt bulbs at 120 volt AC, Aquatic Life brand). The tanks are on a single-pass Fall Creek water flow through system that can be fed any temperature of water available by heating and refrigeration units in the tank system. Tanks were randomly assigned to treatment of fish to avoid tank bias.

Trial Protocol

After being allowed an acclimation period of 1 hour with conspecifics in an adjacent identical behavioral tank, fish were moved into an trial tank and allowed 30 minutes to establish a baseline behavior. After an additional 10 minutes (minute 40), live food (tubifex worms) was introduced via a blind behind the tank by using a sterile test tube containing 30 ml of water at two locations per behavioral tank behind a second blind. At minute 50, a simulated predator was...
dropped abruptly through the aforementioned hole in the plastic tank cover, allowed to enter the water for approximately 3 seconds, then pulled out of sight using a pulley system which was activated by the operator behind the blind. The simulated predator consisted of a white plastic disk (40 cm diameter) with two black eyes drawn on with permanent marker. Conspicuous eye spots are generally treated as threatening stimuli by fishes (Dill, 1974). This disk was attached to a PVC arm that was held above the tank with clear fishing line. This line was connected to a stop outside of the blind set up around the tank to keep it in place between simulated predation events. The operator used gravity to release the predator into the behavioral tank where it hits the water, swings down, and remains under water until the operator pulled back up on the line and reattached it to the stop behind the blind.

There was then a second identical feeding at minute 70, a random time selected to avoid predator-food association (Werner, Gilliam et al., 1983). At minute 90, predator 2 was released and at minute 110 predator 3 was released following the same procedure as the first predator. The fish were observed for 20 minutes to determine if they returned to the baseline behavior that they had individually established in the first 30 minutes of the trial. All trials were recorded with a GoPro Hero 4 camera on a tripod in front of the tank with the red recording light blacked out.

From the behavioral videos, the latency to respond, the duration of a given response, and the modification of response after a simulated predator stimulus were measured. Because I was interested in calculating the overall movement
following the mock predation events, I calculated a total movement response variable by tallying the number of lines crossed between the 8 delineated sections of the tank for two minutes following each mock predation event (Figure 15). The latency to respond and duration of response were measured in seconds (Schjolden, Stoskhus et al., 2005) and the modification of response by location within the tank was recorded as by tally. To discern whether the fish from either treatment were spending more time near the miniature complex structure available, I first calculated a proportion of the time spent near the structure, then divided the proportion by the sum of the total amount of movement for each individual fish recorded after the three predatory events. The result of this division was then available to be used as a response variable in analyses. Finally, to compare foraging between the two treatments, I used a binomial response of whether or not feeding occurred within 2 minutes of prey being introduced. Foraging events were tallied for 2 minutes following each live prey feeding.

Statistics

All statistical analyses were conducted using statistical software R (Team, 2013). Models were evaluated by looking at the residual regression plots to verify appropriate model fit.

To assess the total movement response between simulated predation events for each treatment, a mixed effects negative binomial generalized linear model (GLM.nb) was used in favor of an ordinary linear regression (Bates,
Mächler et al., 2014). This allowed for response variable that have error
distribution models other than normal distribution, which was necessary due to
having zero inflated count data. The model included the three predation events
and treatment as fixed effects. Unique fish ID was included as a random effect to
account for the individual fish in each behavioral trial being compared in relation
to its own response through simulated predator 1, 2, and 3.

The proportion of movement near the small structure within the behavioral
tank was calculated, following a beta-transformation to account for the division of
a rate by a proportion, by using a linear regression (lm) model (ANOVA) with the
proportion of overall movement.

To evaluate feeding behavior a generalized linear model (GLM) was
constructed using a binominal response of whether or not the fish ate at either or
both feedings as the response variable. Treatment, feeding time and tank were
included as fixed effects while fish ID was included as a random effect because,
again, it represented repeat observations of an unique individual. In all cases I
accepted a value of \( p < 0.5 \) as indicative of statistical significance.

**Results**

Fish reared on complex structure moved more often on average (i.e. had a
higher total movement response based on line crosses) than fish reared in bare
hatchery tanks (glm.nb: \( \chi^2 = 6.3, P < 0.0001 \) (Figure 16). Analyses showed this
using a GLM fit by maximum likelihood (Laplace Approximation).
The proportion of movement near the small structure within the behavioral tank was on average the same between the two treatments ($P > 0.05$). Of the analyzed time (2 minutes per fish per predatory event), both treatments spent approximately 30-31% percent of their time in the section of the tank containing the complex structure (Figure 17). On the simple assumption of equal amounts of time spent in each observation cell, I would have predicted that fish would spend about 12% of the time in each cell.

There was no significant difference in the amount of foraging that occurred between treatments ($\text{glm.b } \chi^2 = 0.371, P < 0.0001$). Between both treatments and both feedings events, fish foraged 20% of the time in the 2 minutes following the introduction of live prey.

**Discussion**

By studying the behavior of steelhead from a wild broodstock, we can contribute to answering questions posed by the ongoing Wild Fishes Surrogate Project (Noakes, Schreck et al., 2013). A central goal of the Project is to provide surrogate fish to researchers to be used in tagging and movement studies to provide information on how wild fish move and survive during downstream migration. As a reminder, key factors in this migration include foraging ability, successful smoltification, and predator avoidance (Baynes, Howell et al., 1994; Brown & Laland, 2001). Here, we will discuss behavioral observations that we made on wild broodstock *O. mykiss* juveniles in the context of foraging ability and predator avoidance while they are rearing in freshwater and before they take
their journey to the Pacific Ocean. By measuring the willingness to take risks while foraging, the reactive distance to a perceived threat, the latency to forage after a disturbance, and the use of sites that provide some shelter/cover one can measure the escape behavior by juvenile salmonids (J. W. Grant & Noakes, 1987). These observations may lead to more detailed assessment of our Wild Surrogate Fish when they are nearing their release into a reservoir or river as smolts.

I observed that fish reared with complex structure moved more than their counterparts reared in bare hatchery tanks where movement equaled a fish crossing from one designated section to another within the behavioral tank. This difference was observed across the entire behavioral trial, which included two live feeding events and three simulated predator events. We are inclined to interpret this result as the juvenile salmon being more likely to be attacked by a predator due to higher visibility when moving. But Martel and Dill showed that in the case of juvenile coho salmon (Oncorhynchus kisutch), although common mergansers (Mergus merganser) were more likely to spot more active fish, in the field territorial (more active) juvenile coho feed more and grow faster than other, non-territorial fish after which they spend less time moving which could lower their risk of mortality overall (Martel & Dill, 1995). This implies that movement may not always result in an increased likelihood for mortality.

Both treatments of fish spent equal amounts of time near the miniature version of complex structure present in the behavioral tank, which was about 30% of the time that was analyzed following mock predation events. Seeking
cover is an established predator avoidance tactic that salmonid species employ at different life stages. The time it takes a juvenile salmonid to react to a threat, as well as the way that the fish reacts, can have notable consequences. For example, young-of-the-year brook trout (*Salvelinus fontinalis*) had a decreased reaction distance in areas with high cover and reaction distance was negatively correlated with foraging rate in one of two streams examined (J. W. Grant & Noakes, 1987; Reinhardt & Healey, 1997). In future studies, we would subject fish to this assessment at different points during rearing to evaluate if their use of cover changes over juvenile life stages.

We acknowledge that all movement is not equal. It is well documented in the literature that fear may manifest in several ways in fishes (Domenici, 2010). Fish may freeze, hide or, alternatively, choose active escape depending on environmental circumstances (Ahmed, Seguin et al., 2011). Not only is it possible for fish to respond to threats with different movements, there is evidence that animals do indeed possess the ability to (i) assess their risk of being preyed upon and (ii) incorporate that information into their decision making (Lima, 1998). This means that fish in behavioral trials such as ours could be modifying their behavior with each successive mock predation incident. Although this is a stimulating idea, we did not feel that it was possible with this study design to objectively determine if this was the case in these trials.

The presence of live prey may have also influenced the Wild Surrogate Fishes movements. Something to consider is that we asked these juvenile fish to forage on live prey during the daytime hours when, their parents as juveniles
rearing in the North Santiam River, probably foraged at night. In a natural stream in British Columbia, juvenile steelhead trout were observed to emerge from the substrate only at night to forage to maximize the use of cover (either in the form of physical refugia from predators or in the form of habitats in which predators are inefficient) (M. J. Bradford & Higgins, 2001). This consideration cannot be tested within our study since, although we used natural-origin broodstock offspring, we also fed them in a hatchery environment during daylight hours for the entirety of their rearing. As it has been pointed out, some behaviors can change in as little as one to two generations depending on the trait and the strength of selection on genetics (H. Araki, Berejikian et al., 2008) while learned behavior can occur in a much shorter time span, especially when it deals with something like a predator cue (B. A. Berejikian, Smith et al., 1999). In addition to outward behavior, increased risk of predation on *O. mykiss* has been shown to result in the higher expression of three candidate genes linked to boldness, appetite regulation, and physiological stress responses (Thomson, Watts et al., 2012).

There was no effect of size on foraging behavior and the use of cover (this was because our fish did not differ significantly in length or weight). Previous studies have shown that size can influence foraging behavior and the use of cover. In juvenile coho salmon (*O. kisutch*), fish that used a refuge in the laboratory under a simulated threat were significantly larger than those in a risky habitat. That said, under the threat of predation, both the average growth and the difference in growth between large and small individuals was less than in
control groups (Reinhardt & Healey, 1997). Future research might consider testing *O. mykiss* in a conventional hatchery environment where size classes within a broodstock can vary dramatically in some cases. In our study, because we are rearing wild broodstock fish at very low densities we, along with the hatchery managers, can modulate growth to match our collaborators size-at-release needs.

So far, we have compared our results to natural-origin salmonids. Although this study examined wild broodstock offspring being reared in an experimental hatchery environment, this situation does not represent the commonly debated subgroups of natural-origin salmon and hatchery-reared salmon of the same species. It has been suggested that hatchery fish often choose inappropriate microhabitats (Fausch, 1993). For example, hatchery-reared fish may take up positions mid-stream in order to forage without taking into account the consistently high water velocities and may be found in higher densities than wild fish therefore increasing competition for food or making a predatory event have a greater impact on the population (B. L. Olla, Davis et al., 1998).

In contrast, wild fish often take up positions in eddies and dart out to forage when prey is presented (Tullos & Walter, 2015). This idea was investigated by exposing young farmed, hybrid, and natural-origin Atlantic salmon to an artificial predator in a semi-natural environment with foraging competition present. The study observed that all groups had equal susceptibility to the artificial predator, though it did not answer whether farmed salmon exhibit
a genetically higher susceptibility to predation than natural-origin salmon through risk taking behavior (Solberg, Zhang et al., 2015).

My study showed differences in movement in behavioral trials that included simulated predation and live foraging opportunities presented to wild broodstock steelhead that were reared either with or without complex structure present in their tanks. This is important information for the Wild Surrogate Project since we have not previously observed predator avoidance or foraging behavior pre-release. In future studies, we suggest introducing the juvenile steelhead to the same type of live prey that will be used before the start of the study to increase the chance of foraging and therefore the chance to observe acute latency to resume foraging after a disturbance, such as Brown and Laland did with Atlantic Salmon reared in an enriched environment (2003). A hidden benefit of better habituation to live prey may be a partial mitigation of the deficit that hatchery-reared fish face upon release, when they must make a quick switch from pellet food to live prey or risk mortality.

In addition to fine-tuning behavioral trials like the ones we executed for this study, it is essential that we understand how a conventionally-reared hatchery fish and, if possible, a natural-origin juvenile fish would react to these behavioral trials to establish a context for future trials. By comparing Surrogate Project reared fish to each other and to outside groups of the same species and age, we may better be able to tweak our rearing strategies to further improve the quality of fish we rear.
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CHAPTER 5- GENERAL DISCUSSION

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**Study Overview**

This study used early life history (egg size), fin morphometrics, fish growth, and behavioral trials to gather new information about natural-origin broodstock fish reared as part of the Wild Chinook Salmon and Steelhead Surrogate Project (Noakes, Schreck et al., 2013). By raising juvenile surrogate fish under controlled conditions, I was able to conduct rigorous testing of alternative rearing conditions that will increase the likelihood of developing adequate structural or operational changes in hatcheries to enhance the future management of winter-run steelhead. The hope was that the methods used would, at the very least, provide new insight into the effects of our alternative rearing tactics. We hoped that any observations deemed positive might be applied to future rearing practices either within the Wild Surrogate Project or in conventional rearing hatcheries in Oregon.

**Egg Size Study**

The goal of Chapter 2 was to determine if egg size at time of spawning influences the growth rate of Surrogate Fish. This was done by showing that differences in growth rates of Surrogate steelhead smolts from the North Santiam River during the first year of life can, in part, be explained by differences in egg size at spawning. I definitively showed that eggs within a single female vary significantly in size. In addition, the fish reared from the smallest eggs from an individual female grew faster (measured by length) over the course of the study than fish reared from the largest eggs of an individual female.
The implications from these observations are directly related to the future management of winter-run steelhead. Hatchery rearing relaxes natural selection, allowing fecundity selection to drive exceptionally rapid evolution of small eggs and, in addition, trends toward small eggs are evident in natural populations heavily supplemented by hatcheries, even when female body size stay the same (Heath, Heath et al., 2003). Growing quickly is a complicated phenotype. It can influence the amount of agonistic behavior displayed, with larger fish being more aggressive and setting up larger territories (B. A. Berejikian, 1995), but also more likely to forage under threat of a predator in the hatchery environment (Johnsson, 1993). This information should be kept in mind as attempts are underway to rebuild the natural-origin *O. mykiss* population in the North Santiam River using hatchery supplementation.

**Structure and Fin Quality**

Chapter 3 aimed to determine if artificial in-tank structure improves surrogate fish quality over a 6-month period. The main hypotheses stated that fish reared in the presence of complex structure would, a) better mimic a natural growth trajectory than a fish reared in an average hatchery environment and b) have a higher fin quality (less fin degradation) than fish raised in bare tanks, at age of release. To quantify this, monthly sampling was conducted to assess morphology of the dorsal and caudal fins as well as growth. Fish reared with complex structure had longer anterior dorsal fins at the final sampling event, but dorsal fin length did not differ significantly at other points in the study. The dorsal
lobe of the caudal fin was longer in fish reared with structure, and the caudal fin in relation to body length ratio was found to vary between treatments, indicating asymmetry. Fish reared with complex structure grew at the same rate as fish without complex structure over the course of this study. Fins deteriorated at the same rate between small and large fish in both treatments, which could show an effect of allometric growth.

Growth in a conventional hatchery environment often appears linear with fish achieving maximum growth in a given time frame, but in this study we observed a growth pattern that better followed a natural-origin growth trajectory. By allowing growth rates to vary, we allow Wild Surrogate Project fish to better mimic their natural-origin counterparts rearing in streams. Fin quality is easily quantified as an indicator of overall fish health (T Ellis, Hoyle et al., 2009) and fin erosion in the hatchery environment is a well-known effect of fish rearing density (North, Turnbull et al., 2006) and may be a factor in reducing fish survival (Bosakowski & Wagner, 1995; Latremouille, 2003). Less dorsal fin degradation has been observed in juvenile steelhead reared in conventional hatchery tanks, enriched tanks, and a natural stream but all were reared at a higher density than in our study (Barry Berejikian, 2005). Not only is there evidence that complex structure in the rearing environment may improve fin quality, there is also evidence that it may improve feeding rates of juvenile salmon on natural prey in salmonid species (Rodewald, Hyvärinen et al., 2011).
Complex Structure and Movement

In concert with Chapter 3, Chapter 4 asked if artificial in-tank structure has an influence on the foraging and/or predator-avoidance behavior of Wild Surrogate fishes near their date of release. In behavioral trials, fish reared on complex structure moved more often on average (aka had a higher total movement response) than fish reared in bare hatchery tanks. The proportion of movement near a small structure within the behavioral tank was on average the same between the two treatments (approximately 30-31% of the time) in the section of the tank containing the complex structure. There was no significant difference in the amount of foraging that occurred between treatments. Between both treatments and both feedings events, fish foraged 20% of the time in the 2 minutes following the introduction of live prey.

Studying the behavior (foraging ability and predator avoidance) of steelhead from a wild broodstock allowed me to contribute to new questions posed by the ongoing Wild Fishes Surrogate Project (Noakes, Schreck et al., 2013). By measuring the willingness to take risks while foraging, the reactive distance to a perceived threat, the latency to forage after a disturbance, and the use of sites that provide some shelter/cover I could measure the escape behavior by juvenile salmonids (J. W. Grant & Noakes, 1987). Appropriate escape behavior could help a juvenile salmonid survive a migration through a dam, estuary, or back to its natal spawning grounds as an adult.
Study Implications and Future Concerns

Studies like this one strive to better understand the differences between hatchery and natural-origin salmonids through rearing wild broodstock offspring in a modified hatchery environment. The early and juvenile life history stages are important because it has been shown that young hatchery-origin fish released into the wild survive at much lower levels than their wild-counterparts (Kostow, 2004), although hatchery-reared juvenile salmon do not always exhibit higher mortality (Rhodes & Quinn, 1999), and survivors of the two groups have been shown to experience more similar survival in the ocean environment (Unwin, 1997). Those results have raised concerns for many involved in both the recovery of wild salmonids as well as the release of hatchery fish, including hatchery managers, tribes, anglers, conservation groups, and the public. Concerns and politics concerning hatchery rearing started as early as the first hatcheries were established. As Foerester articulated, “To introduce for consideration the question of the value of and benefits derived from artificial propagation of salmon as compared with natural propagation may appear to many as the unearthing of a well-gnawed bone of controversy, but it must be remembered that there is still lacking that definite, concrete, scientific information on which an argument should be properly based.” He continued to describe the brewing fight between fish culturists and scientists who were skeptical of the romantic picture being painted (1931).

Recently, it has been demonstrated that the reproductive fitness decline due to captive rearing can occur as quickly as over one or two generations (H.
Araki, Berejikian et al., 2008). Those authors also stated that if selection acts on a single trait, such rapid effects can be explained only when selection is very strong, both in captivity and in the wild, and when the heritability of the trait under selection is high. This is new information gives context to the concerns of the scientists of the early 20th Century and provides support for research today. This evidence makes waiting to take action in modifying rearing practices or protecting natural-origin populations a dangerous prospect when dealing with a federally threatened group of fish, as we are.
Literature Cited


FIGURES AND TABLES
Figure 1. The North Santiam River is a 92-mile (148 km) tributary of the Santiam River in western Oregon, USA. It drains 766 square miles (1,980 km²) of the Cascade Range on the eastern side of the Willamette Valley east of the state’s capital city of Salem. There are two dams, Big Cliff and Detroit at river mile 46 and 49, respectively, which produce a combined output of almost 120,000 kW of hydroelectricity. Just downstream of these dams is Minto Fish Collection Facility, owned and operated by the USACOE.
Cameron Sharpe of ODFW taking samples from wild *Oncorhyncus mykiss* female broodstock. Samples include scales, genetic fin clips, head kidney, and ovarian fluid.

*In vitro* fertilization procedure of steelhead gametes. Pictured are female eggs and male milt being fertilized in a mesh container before being submerged into fresh water.
Figure 3. Determining Egg Size

Figure 3. A sample of Female 4’s embryos to be measured digitally to determine the range of her egg size.
Figure 4. Cartoon depiction of .tpsDig Software used to Determine Egg Size
Figure 5. Distribution of Egg Size by Female

Figure 5. Female 2 and Female 7 were excluded from the study after it was determined that their eggs were statistically smaller on average and larger on average and, therefore because the large and small eggs would be pooled for the duration of the study, it was impossible to include them in the groups. Inclusion may have caused a disproportionate skew in the later growth data by giving the smallest and largest female an unfair amount of representation.
Figure 6. Growth of All Tanks

Figure 6. Average growth of fish in all tanks over the course of the study representing monthly sampling. At the end of the study, the small-origin fish reared in a mixed treatment were significantly larger than fish in the small-egg origin tanks grew to a significantly greater mean length than fish in the large-egg origin tanks ($F_{(1,94.66)}=0.008 \ p = 0.006$). At ponding, the large-egg origin fish were slightly (48.2 cm) but not significantly longer in fork length than small-egg origin fish (47.3 cm). Small-egg origin fish from the uniform treatment grew longer than the large-egg origin fish from their uniform rearing treatment ($F_{(1,48)}=0.033 \ p = 0.06$).
Figure 7. Small-egg origin fish from the uniform treatment grew longer than the large-egg origin fish from their uniform rearing treatment ($F_{(1,242.3)}=0.033 \ p = 0.06$) (Figure 4). Between month three and four (October and November 2014), the small-egg origin fish length, on average, surpassed the large-egg origin length. This trend was maintained through day 213 (March 2015) when the project was terminated.
Figure 8. A single sampling day (212) that represents a trend seen throughout the study of fish reared in a mixed treatment (50% small-egg origin fish, 50% large-egg origin fish) are longer on the day sampled than their counterparts reared in isolated treatments. N = 2 tanks each for large and small groups and n = 1 tank each for large mixed and small mixed groups.
Figure 8. This is one sampling day (212) but it represents a trend seen throughout the study of small-egg origin fish being longer on the day sampled than their counterparts from large-egg origin fish, irrespective of treatment.
Figure 10. Photograph of Surrogate Fish Using Complex Structure
Figure 11. Nine Morphometric Landmarks Used in Fin Quality Analysis

![Image of a fish with numbered landmarks]

Figure 12. View of Single Behavioral Tank with Complex Structure

Figure 14. Photograph of single behavioral tank with complex structure pictured and juvenile test fish in foreground.
Figure 13. Diagram of Designated Tank Sections used in Behavioral Trial Analyses

Figure 15. Cartoon depiction of the 8 designated sections used to count movement by Surrogate Fish in behavioral trials. A cross of one line equaled one movement in the trials.
Figure 14. Total Movement of Fish

Figure 16. Fish reared on complex structure moved more often on average (i.e. had a higher total movement response based on line crosses) than fish reared in bare hatchery tanks (glm.nb: $\chi^2 = 6.3$, $P < 0.0001$).
Figure 15. Proportion of Movement Near Complex Structure

Figure 17. The proportion of movement near the small structure within the behavioral tank was on average the same between the two treatments (P > 0.05). Of the analyzed time (two minutes per fish per predatory event), both treatments spent approximately 30-31% percent of their time in the section of the tank containing the complex structure.
Table 1. Table of Largest and Smallest Egg Size Quartiles by Female

<table>
<thead>
<tr>
<th>Female</th>
<th>First Quartile</th>
<th>Third Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.125525</td>
<td>0.13815</td>
</tr>
<tr>
<td>3</td>
<td>0.12265</td>
<td>0.133425</td>
</tr>
<tr>
<td>4</td>
<td>0.12695</td>
<td>0.13435</td>
</tr>
<tr>
<td>5</td>
<td>0.128925</td>
<td>0.138575</td>
</tr>
<tr>
<td>6</td>
<td>0.12345</td>
<td>0.129275</td>
</tr>
</tbody>
</table>

Table 1. Quartiles of largest and smallest eggs (g) per female that remained in the study. These weight cutoffs were used in weighing of each individual female’s eggs to gather from her largest and smallest size class.
Table 2. Average Lengths of Surrogate Fish at End of Growth Study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. Length (mm)</th>
<th>Range</th>
<th>SE (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>92.27</td>
<td>46</td>
<td>1.5</td>
</tr>
<tr>
<td>No Structure</td>
<td>94.42</td>
<td>56</td>
<td>1.83</td>
</tr>
</tbody>
</table>
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